CHEMICAL INVESTIGATION OF SOME INDIAN MEDICINAL PLANTS

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This dissertation embodies the results of chemical studies on two plants, namely Physalis franchetii Mast. and Anacardium giganteum Hancock ex Engl. and has been divided into two chapters. Preceding the discussions of the chapters is the "Theoretical", which highlights the recent advances in the analytical techniques applied to the isolation and structure elucidation of the steroids. It also includes the list of stigmastane type of compounds isolated and characterised during the period 1958 to 1971, which have been classified and placed in six tables along with the plants of their origin.

Chapter I is concerned with the chemistry of sterols isolated from the seeds of Physalis franchetii Mast. The plant is reputed to be a diuretic in the indigenous systems of medicine. The hexane extract of powdered seeds of the plant, on chromatography yielded β-sitosterol and two unknown sterols designated as physanol A and physanol B.

Physanol A was obtained as fine colourless needles from chloroform-methanol, m.p. 234-36°, $[\alpha]_D$ = 60° (C 1 %, chloroform), $C_{36}H_{50}O_4$ (M^+ 546). Its colour reactions

indicated a steroid nature. The IR spectrum of physanol A revealed the presence of OH group, aromatic nucleus, CH_3-, $-CH_2-$, aromatic ester grouping, enone group and a $>C=CH_2$ group. The presence of a benzoyloxy group was indicated by the UV absorption at 237 nm. Its PMR spectrum manifested signals for two quaternary methyls, two secondary methyls, $-\underset{|}{C}\underline{H}-O-$, $-\underset{|}{C}\underline{H}-O-CO-$, $>C=C\underline{H}_2$, $-\underset{|}{C}=C\underline{H}-CO-$ and $-C_6\underline{H}_5$. It readily formed a monobenzoate, m.p. 210-11°, $C_{43}H_{54}O_5$ (M^+650) and a monoacetate, m.p. 204-5°, $C_{38}H_{52}O_5$ (M^+588). The PMR spectrum of mono-O-acetyl physanol A displayed one acetyl singlet at 2.0 ppm and a 1H doublet of multiplets for -CHOAc at 5.25 ppm, but no hydroxyl absorption in the IR spectrum. This indicated the presence of only a secondary hydroxyl group in physanol A.

Since the IR and PMR spectra of physanol A indicated the presence of a vinylidene group, it was treated with m-chloroperbenzoic acid to form a monoepoxy derivative, m.p. 225-30°, $C_{36}H_{50}O_5$ (M^+562). Its PMR spectrum was lacking the vinylidene proton signals, instead it showed a 2H multiplet at 2.68 ppm assignable to methylene protons on one of the carbons bearing the epoxide linkage. On hydrogenation of physanol A in glacial acetic acid in presence of Adam's catalyst, the dihydro as well as the tetrahydro derivatives were obtained in a ratio of about

8:1. In dihydrophysanol A, m.p. 232-33°, $C_{36}H_{52}O_4$, (M^+ 548) only the vinylidene double bond was reduced as indicated by its IR and PMR spectra, whereas in the tetrahydro derivative of physanol A, m.p. 128°, $C_{36}H_{54}O_4$ (M^+550), both vinylidene as well as the conjugated double bonds were reduced.

The molecular formula of the physanol A ($C_{36}H_{50}O_4$) and the products of its saponification ($C_{29}H_{46}O_3$, vide infra) in conjunction with its physicochemical data suggested that physanol A was the benzoate of a C_{29} steroid (stigmastane type). The nature of the side chain as stigmastane type was also revealed by a prominent loss of 139 mass units corresponding to the elimination of $C_{10}H_{19}$ side chain in the mass spectra of physanol A and its debenzoylated derivatives. In the mass spectra of dihydrophysanol A as well as tetrahydrophysanol A, there was a corresponding loss of 141 mass units ($C_{10}H_{21}$) as in the case of β-sitosterol. Thus the basic skeleton of physanol A was considered to be of stigmastane type.

The saponification of physanol A furnished, besides benzoic acid, two products designated as debenzoylphysanol A and debenzoylphysanol A_1 in order of increasing Rf values. Debenzoylphysanol A, m.p. 209°, $C_{29}H_{46}O_3$ (M^+442) contained all the functional groups as present in physanol A except that the benzoate group was hydrolysed to a hydroxyl group

suggesting that this was the normal product of saponification of physanol A. The other product, debenzoylphysanol A_1, m.p. 210-11°, $C_{29}H_{46}O_3$ (M^+442), did not contain any enone group (UV, IR). Its PMR spectrum showed the presence of a 2H signal for-CO-CH_2-C=C, but none for vinylic protons indicating that the double bond in this compound originally in conjugation with the carbonyl group, took up a tetra-substituted position during alkaline hydrolysis of physanol A.

Since the vinylidene group was present in the side chain (Mass), it could only be assigned Δ^{20} position which accounted for a pair of 1H singlets at 4.86 and 4.95 ppm, in the PMR spectrum of physanol A. The fact that the conjugated double bond in physanol A was capable of migrating to a tetrasubstituted position in alkaline medium narrowed down the location of the enone group (in a six membered ring) either to $\Delta^{9(11)}$, 12-OXO or Δ^{7}, 6-OXO positions. However, the splitting pattern (quartet J = 1.5 Hz) of the PMR signal of a vinylic proton adjacent to the carbonyl group, arising from its coupling with two allylic protons on γ-carbon atoms, clearly indicated that the enone group occupied the Δ^{7}, 6-OXO position.

The oxidation product of physanol A (ketophysanol A) displayed the IR absorption at 1703 cm^{-1} (six membered ring ketone), showing that the hydroxyl group was present

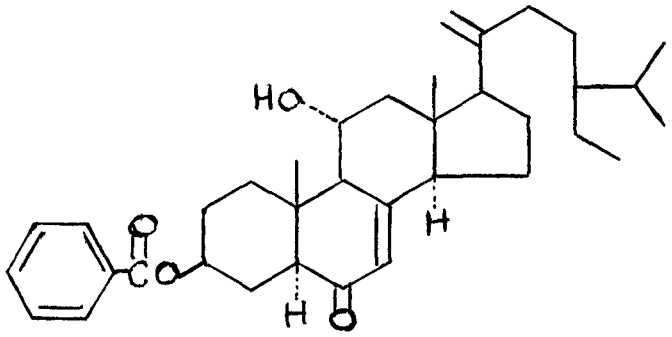
in the six membered ring. Further, the PMR spectrum of keto physanol A showed a 2H singlet at 3.11 (C-12 methylene) and another sharp singlet at 2.1 ppm, (probably 1H, C-9 methine) suggesting C-11 as the position of the keto group. The hydroxyl group could thus be located at C-11. This was assigned an α-equatorial configuration on grounds of its easy acetylability and the splitting patterns (dm, J = 10 Hz) of the C-11 methine proton in the PMR spectrum.

Since the debenzoylated products of physanol A did not form an acetonide and were also inert to the action of sodium periodate, the possibility of 1,2 diol grouping in these products was ruled out. The PMR spectrum of physanol A had a smeared type broad multiplet at 4.66 ppm representing a proton adjacent to the benzoyloxy group, which on debenzoylation shifted downfield to 3.13 ppm (in the PMR spectra of debenzoylphysanol A and A_1). The position and shape of this methine multiplet in the PMR of debenzoylphysanol A and A_1 was very characteristic of 2β-axial or 3α-axial proton of 2α or 3β hydroxy steroid respectively. In view of the ubiquitous presence of an oxygen function at C_3 in natural steroids, the benzoyloxy group was considered to be present at C-3 position.

The placement of functional groups in physanol A was further confirmed by calculating the shift values of

18-methyl and 19-methyl groups of physanol A and its various derivatives taking 5<-stigmastane as the basic skeleton and comparing these values with the corresponding observed values. The calculated values were found to be in excellent agreement with the observed values.

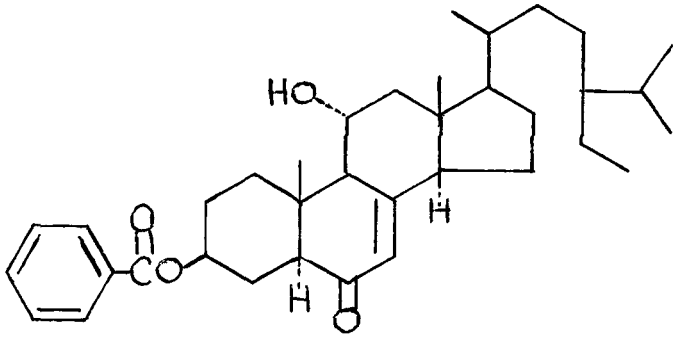
On the basis of above evidences structure (I) was proposed for physanol A.



(I)

Physanol B, m.p. 232-33°, $[\alpha]_D$ = 68°, $C_{36}H_{52}O_4$ (M^+548). The colour reactions and physicochemical data of physanol B were very similar to those of physanol A and indicated the presence of all functionalities of the latter except the vinylidene group. It was, therefore, concluded that physanol B was the dihydro derivative of physanol A. It was confirmed by superimposable IR spectra, mixed melting points and Co-chromatography (TLC) of physanol B

with dihydrophysanol A.) The structure of physanol B was, therefore, proposed as (II).



(II)

(The identity of compound C, m.p. 136-37°, was confirmed as β-sitosterol from its mixed melting point, superimposable IR spectra and co-chromatography with an authentic sample.)

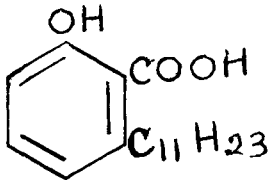
Chapter II deals with the chemical investigation of Anacardium giganteum Hancock ex Engl. (The hexane extract of the pericarp of the mature nuts yielded a crystalline phenolic acid, designated as anagigantic acid. It was crystallised from hexane in the form of stars and aggregates of silky needles, m.p. 81°, $C_{18}H_{28}O_3$. The IR spectrum of anagigantic acid manifested the presence of aromatic nucleus, CH_3-CH_2-, Ar-COOH.)The PMR spectrum of anagigantic acid exhibited signals for a terminal -CH_2-CH_3 group, -$(CH_2)_9$-, -CH_2-CH_2-Ar, three adjacent aromatic protons and hydrogen bonded hydroxyl protons. Anagigantic acid, gave

a violet like colouration with ferric chloride indicating the presence of a phenolic group. Methylation of the compound with diazomethane yielded a colourless viscous liquid which gave a violet colouration with ferric chloride, but was insoluble in dilute alkali, showing partial methylation of the carboxylic group. Methyl anagigantate was further methylated with dimethyl sulphate and aqueous potassium hydroxide to yield methyl ether of methyl anagigantate which gave no colouration with ferric chloride and was found to be insoluble in dil. alkalies.

From the above observations it was obvious that anagigantic acid was an aromatic carboxylic acid having a long residual $C_{11}H_{23}$- chain and a phenolic group. It yielded lauric acid on potassium permanganate oxidation in alkaline medium. The formation of this acid must have resulted from the splitting of the aromatic nucleus and thus the simplified formula for anagigantic acid could be written as (C_6H_3), (OH), $(COOH)$, $(C_{11}H_{23})$.

In order to fix the orientation of the hydroxyl and carboxylic groups and the $C_{11}H_{23}$- chain on the benzene nucleus, oxidation of methyl ether of methyl anagigantate was carried out with potassium permanganate in aqueous pyridine yielding 3-methylphthalic anhydride indicating

that the carboxylic group and the undecyl chain were in adjacent positions. Further, the hydroxyl group and the undecyl chain were proved to be present in meta position to each other by decarboxylation of the anagigantic acid to anagigantol, followed by methylation and finally the oxidation of the resulting methylether to m-methoxybenzoic acid. The structure of anagigantic acid could thus be written as (III).



(III)

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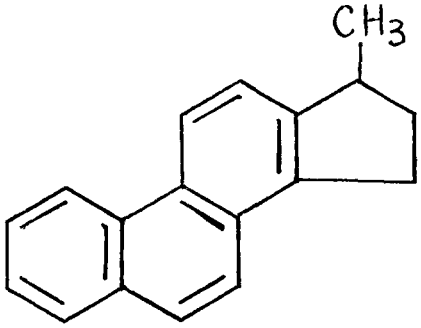
C O N T E N T S

THEORETICAL

The steroids as a whole comprise a wide group of natural products possessing a tetracyclic carbon skeleton of cyclopentenoperhydrophenanthrene. All the steroids on selenium dehydrogenation at 360° yield among other products Diels' hydrocarbon(I). A steroid may, therefore, be defined as any compound which yields Diels' hydrocarbon on selenium dehydrogenation.



(I)

Steroids occur in a wide variety of organisms in the animal as well as in the plant kingdom. The diverse type of steroidal compounds arise mainly from the variation in the number of carbon atoms and the nature of the side chain, apart from the minor differences appearing due to nuclear substituents and the degree of unsaturation. Steroid types are listed in Table 1 along with the nature of the side chain and important examples of each.

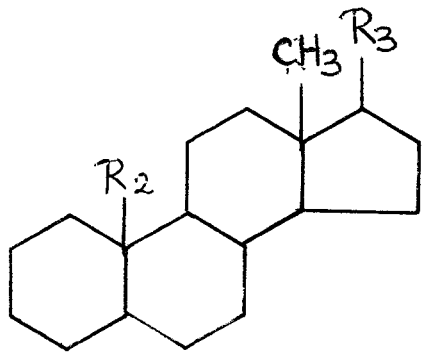


TABLE 1 : Classification of Steroids

No. of C atoms	Type	R_2	R_3	Examples
18	Oestrogens	-	-	Oestradiol, Oestrone Equilenin.
19	Androgens	CH_3	-	Androsterone, Isoandro-sterone, Testosterone, Rubrosterone.
21	Gestogens	CH_3	$-CO.CH_3$	Progesterone.
21	Adreno-cortical hormones	CH_3	$-CO.CH_2OH$	Deoxycorticosterone, Corticosterone, Cortisone.

Contd.....

TABLE 1 : (Contd....)

No. of C atoms	Type	R_2	R_3	Examples
21	Cardiac aglycone	CH_3	β-Ketotetrahydrofuran ring	Digenigenin
23	Cardiac aglycones	CH_3	$\overset{\alpha\beta}{\Delta}$-$\gamma$-lactone	Periplogenin,
		CH_2OH		Ouabegenin,
		CHO		Strophanthidin
24	Cardiac aglycones	CH_3	$\overset{\alpha\beta:\gamma\delta}{\Delta}$-$\delta$-lactone	Scillaren A,
		CHO		Hellebrin
24	Bufotoxins(Toadpoison)	CH_3	-	Bufalin, Telocino-bufagin, Gamabufotalin
24	Bile acids	CH_3	$-\overset{CH_3}{\underset{\vert}{C}}H-(CH_2)_2COOH$	Cholic, Deoxycholic, Lithocholic acids etc.
27	Sapogenins	CH_3	C_8-Spiro-Ketal system	Sarsasapogenin, Tigogenin, Diosgenin etc.
27	Zoosterols	CH_3	$-\overset{CH_3}{\underset{\vert}{C}}H-(CH_2)_3-CH(CH_3)_2$	Cholesterol, Coprostanol

Contd.....

TABLE 1 : (Contd....)

No. of C atoms	Type	R_2	R_3	Examples
27	Insect Hormones	CH_3	$-\dot{C}(CH_3)(OH).CH(OH).CH_2.CH_2.CH.(CH_3)_2$	Ponasterone A, Ponasterone B.
28	Mycosterols	CH_3	$-\dot{C}H-(CH_2)_2-\dot{C}H.CH.(CH_3)_2$	Ergosterol, Chalinasterol
	Marine sterols Phytosterols			Brassicosterols, Campesterol
28	Insect moulting hormones	CH_3	$-\dot{C}(OH).CH(OH).CH_2CH(CH_3).\dot{C}.(CH_3)_2$ (OH)	Makisterone A
			$-\dot{C}(OH).CH(OH).CH_2CH(CH_3).CH(CH_2OH)$ ($\dot{C}H_3$)	Makisterone B
29	Phytosterols	CH_3	$-\dot{C}H-(CH_2)_2-\dot{C}H-CH.(CH_3)_2$ (C_2H_5)	Stigmasterol
29	Insect moulting Hormones	CH_3	$-\dot{C}(OH)-CH(OH).CH_2.CH(C_2H_5).-\dot{C}.(CH_3)_2$ (OH)	Lemmasterone
			$-\dot{C}(OH).CH(OH).CH_2-CH(CH_2)_2OH.CH(CH_3)_2$ (CH_3)	Amarsterone B

Of these types of steroids the interest of author, has mainly been with the phytosterols, especially C_{29} type (Stigmastane type). Since 1930 the phytosterols have aroused much interest as they were found to serve as excellent starting materials for the preparation of sex-hormones and vitamin D, e.g. ergosterol could be converted to vitamin D and stigmasterol to progesterone. Various reviews and books have been published from time to time covering steroid as a whole or only of a particular type in this class[1]. The most exhaustive treatise in this field available so far is "Steroids" by L.F. Fieser and M. Fieser[2] which was published as back as 1958. The present review has, therefore, been envisaged to highlight the advancements in the field of steroids during the period 1958-71.

Analytical Techniques :

The isolation of a pure substance is frequently the major laboratory effort required for tackling a constitutional problem pertaining to a natural product. In this attempt one or more chromatographic methods may usually be employed. The pure product is then subjected to various physicochemical studies and chemical degradations. The modern physicochemical techniques include molecular-rotation, ultraviolet, infra red and nuclear magnetic resonance spectroscopy, mass spectrometry,

molecular mass spectrography, optical rotatary dispersion, circular dichroism and x-ray crystallography. A brief account of all these techniques is given below with special emphasis on their recent applications in the field of natural steroids.

Chromatography :

The isolation of steroids from the natural sources presents great difficulty because of their close chemical relationship and their notable tendency to form molecular complexes and mixed crystals. Chromatographic techniques have proved a powerful tool in the hands of chemist for handling such problems effectively. Paper, thin layer chromatography and column chromatography are now used as routine techniques for detection and isolation of steroids.

The concept developed by Bate-Smith and Westall[3] that the Rf values for the components of a molecule contribute additively to its paper chromatographic mobility has also been applied to steroid analysis[4,5] and is useful in characterisation of unknown steroids. Recent modifications[6-8] in the chromatographic techniques such as the use of silica gel impregnated with silver nitrate for unsaturated steroids[9] and dry column chromatography have enhanced the efficiency of separation and purification manyfolds. In dry column chromatography the columns are packed with the

dry absorbents and developed as the thin layer plate until the solvent front has reached near the end of the absorbent column. The developed column may then be extruded and desired zone eluted[10]. A similar procedure using inverted columns has also been recommended[11]. A somewhat more convenient procedure is the use of nylon tubes for chromatography. The thin wall tubing transmits UV light and by incorporating a fluorescent mineral such as zinc silicate in the adsorbent, UV absorbing materials may be detected. The column is then sectioned and the desired zone eluted. The use of dry column has the potential advantage that the thin layer chromatography can be used to standardise a solvent system giving the desired separation. Thus the evaluation of various solvent systems can be done within a short time and with a small amount of material. The steroids of higher polarity have been resolved on the columns of ion-exchange resins[12]. The separation of steroids has also been achieved by Gel-chromatography on methylated Sephadex[13,14] in organic solvents.

Gas-liquid chromatography is another leap forward in the techniques of separation and is endowed with a unique feature that after completion of one analysis, the system is immediately ready for the next. It is an extremely powerful method for separating the mixtures by partitioning the components between a flowing gas and a stationary liquid

phase supported on an inert solid packed into a column. The material to be separated is flashed into the carrier gas which flows through the column maintained at a constant temperature below the boiling points of liquid phase. The components of the mixture move through the column at different rates depending on their partition co-efficient between gas and the liquid phases and emerge separately into the gas stream to be detected or if required, collected. The use of gas chromatography in the analysis of a sterol mixture by itself or as a supplement to other methods of separation may yield more fruitful results then have been possible uptil now. The relative simplicity with which a very effective separation can rapidly be obtained is a great advantage of this technique. The most important use of GLC lies in the identification of unknown substances and determination of their relative amounts present in a sample. With simple steroids at least sharp peaks are obtained and closely similar compounds, e.g., C_5-epimers may be resolved[15-20]. Steroid sapogenins[21] and various steroid hormones[22-24] have been successfully resolved.

The large molecular size and diversity of substitution of natural steroids frequently pose a variety of problems in GLC due to their adsorption on the chromatographic column, ease of dehydration and decomposition or lack of volatility. A pre-treatment of the steroid material leading to suitable

volatile derivatives with altered relative retention time is, therefore, a desirable step prior to GLC. Thus the polyhydroxylated compounds are converted into their acetates, propionates, trifluoroacetates[25], methylethers and trimethylsilylethers[26]. The latter are especially preferred because of the simplicity of their preparation even with the microquantities of the sample. Acids are generally converted to their methyl esters. The fluoro-alkylsilicone polymer QF-1-0065 has been found to be a particularly suitable stationary phase for the GLC of steroids[27,28]. It exhibits a greater affinity for ketones than for alcohols. Interestingly, the use of steroid derivatives viz., cholesteryl benzoate, p-phenylbenzoate and 5α-cholestane-3β-yl benzoate as stationary phase in gas liquid chromatography has also been reported for the separation of steroids[29].

The qualitative property of the solute that defines its mobility is expressed in terms of retention time which, however, is dependent on a given set of conditions such as temperature, column length, column diameter, type and amount of the liquid phase etc. The retention time is, therefore, commonly calculated relative to some other standard compound. This relative retention time, denoted by γ, is independent of the above conditions except the liquid phase

and the column temperature. Clayton[30] studied the correlation of retention time with the structure of steroids in GLC. During these studies he observed that the more hindered axial methoxyl group rendered the compound more mobile than its epimer. In case of C_{30} steroids, the 4,4-dimethyl grouping hindered the 3β-oxygen function, resulting in the lowering of the retention time as compared to stigmastanol type of compound. Steroid having nuclear double bonds showed lower retention time than the corresponding saturated compound. The Δ^7 bond was found to exert the greatest effect of any isolated double bond. The homoangular 5,7-diene enhanced the retention time still further. The double bond at C_{24} in the side chain increased the retention time but Δ^{22} double bond showed an anomalously low retention time. This was attributed to the restricted movements of the side chain. The above correlative studies suggested[31] that in general the introduction of a substituent group in a specific position of a steroid changes the retention time by a factor which is constant for the type of grouping and its particular position and is also independent of molecular weight of the steroid and of the influence of other functional groups. Thus the retention time (r) of a polysubstituted steroid in which intramolecular group interactions are negligible could be expressed as :

$$r = r_a \times K_a \times K_b \times K_c \text{ -------}$$

where r_n is the retention time of the unsubstituted nucleus and K_a, K_b, K_c-- are group retention factors for a series of groups at position a, b, c-of the nucleus.

Knight and Thomas[32] have shown that the logritha of the relative retention time (log r) of a steroid is made up of the additive contributions of the substituents together with that of the steroid nucleus to which they are attached. The change in log r due to simple substitution of hydrogen by another group is denoted as $\triangle$ Rmgr, while the change in log r resulting from a simple chemical reaction such as reduction and epimerisation etc. is referred to as $\triangle$ Rmr value. The predetermined $\triangle$ Rmgr and $\triangle$ Rmr values can be used for the systematic characterization of the unknown steroids. Thus the LAH reduction of various ketosteroids to the corresponding hydroxy derivatives was carried out and the products were analysed by GLC on 6 % QF-1 as the stationary phase[33]. The $\triangle$ Rmr values for reduction of keto groups at different positions of the steroid nucleus were determined ; 3-OXO-(-0.27), 17-OXO-(-0.19) and 3-OXO-$\triangle^4$ -(-0.8 to 0.9). These values were applied for locating the position of keto group in the unknown steroids. The reduction of 3-ketones is stereospecific giving only equatorial hydroxyl. The 17-ketone are also reduced to 17β-hydroxy compounds only. Thus the GLC of these reduction

products show only one peak and can easily be identified. They can further be distinguished from the 20-OXO-steroids by the fact that the chromatogram of the reduction product of the latter group of compounds show two peaks, associated with the epimeric 20-hydroxy steroids. Alternatively, a 20-OXO-17β-steroid can be epimerised to 20-OXO-17α-steroid by treatment with alkali and then its $\triangle$Rmr value measured, which is -0.08. Thus these simple chemical reactions perform a function equivalent to that of specific colour tests in the paper chromatography.

The $\triangle$Rmr values for the differences in configurations are generally of small order. In case of C_5-isomers $\triangle$Rmr value is influenced by the nature of substituent at C_3. Acetylation of an equatorial 3-hydroxy group has a marked effect on $\triangle$Rmr value of a change in configuration at C_5. This also demonstrates the potentialities of esterification for separating the isomers. Thomas et al.[33a] have made an excellent use of the above methods for the identification of steroid hormones and their metabolites in human and rhesus pregnancy urine.

The deuteration of the steroids having readily exchangeable protons, such as hydroxylic proton can also be performed by GLC with a suitable stationary phase, e.g., polyethylene glycol saturated with D_2O. The enolic hydrogen atoms have been replaced effectively by deuterium in such columns

only in presence of strongly acidic or basic catalysts. Columns consisting of 1 % Apiezon-L containing 0.5 % KOH on gas chrome-Q have been found to be suitable for deuteration of steroid ketones. This method has been applied to the characterisation of saturated and unsaturated mono-steroids by combined GLC-mass spectrometry[34].

Optical Rotation :

Optical rotations have been of great utility in the structure elucidation of steroids. Since their molecules are fairly flat and rigid and functional groups are also often widely separated, the rules of optical superposition holds good with a fair degree of accuracy in these compounds. The correlation of steroid structure and optical rotation was first initiated by Young et al.[35]. It was then extended by Barton[36,37] and Klyne[38]. The contributions of certain functional groups to the molecular rotations of steroid are characteristic of the positions of their substitution and are independent of one another. However, if highly unsaturated groups, e.g., α,β-unsaturated keto groups are present or if other groups are too closely situated, they may influence each others rotational contributions and exhibit "vicinal action". Thus such information regarding the substituent groups in a steroid can be elicited from the optical rotations of the compounds. It has also been possible to predict the

sign and magnitude of optical rotation by conformation analysis of steroids[39].

Further, Bose[40] has given an empirical rule relating the relative molecular rotations of epimeric α-substituted cyclic ketone with the configuration of the more and the less bulky α-substituents.

Ultraviolet Spectroscopy :

The principal UV absorption maximum and the molecular extinction coefficient (ε) can often be correlated with structural features of a steroid. The empirical rules for the predictions of the absorption maxima deduced by Woodward[41] have successfully been applied by Fieser and Fieser[2] in relation to the structure of steroids.

A relatively recent application of UV spectroscopy has been made in settling the position of enone chromophores in various insect moulting hormones[42]. The UV absorption of the steroids in the lower wave length region down to 187mμ have been studied in order to distinguish between the isolated double bonds having various degrees of substitutions[44]. The position of the UV low intensity carbonyl absorption has been found to be decisive in 4,6-disubstituted Δ^4-3-ketones[45].

Infra-Red Spectroscopy :

Steroids constitute an ideal class of compounds for infra-red spectroscopic studies, since they provide a large group of related substances with a fairly rigid fused ring system with a definite stereochemistry. Most of the work in this field has been concerned with the characterisation of hydroxyl and carbonyl groups, determination of configuration of certain positions and locating the centres of unsaturation in the molecule. A wealth of information on the spectra-structure correlations of steroids is available in the literature[46-49].

Recently, the position and shape of the hydroxy bands have been studied in relation to the structure of steroids[50]. Further, the C-O- stretching vibrations of the C-OH grouping have also been studied[51] in the 3-hydroxy steroids with unsaturation in ring B. For Δ^7, the C-OH band appears at standard position (1040 cm^{-1}) whereas for Δ^5 it appears at (1050 cm^{-1}). A 7-OXO- Δ^5- grouping shifts the band to 1057 cm^{-1}. In case of a 5,7-diene two bands of comparable intensities appear at 1053 and 1038 cm^{-1} for equatorial and axial hydroxyl group respectively.

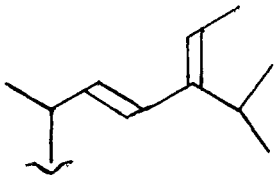
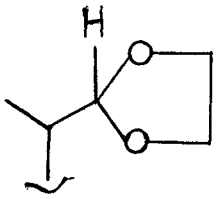
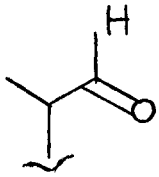
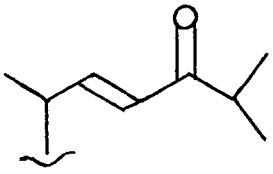
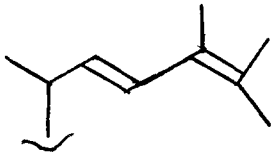
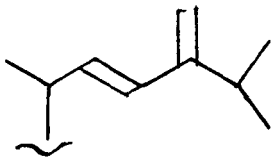
Nuclear Magnetic Resonance Spectroscopy :

During the last decade the nuclear magnetic resonance spectroscopy has emerged as one of the most powerful tools in the structural elucidation of steroids. Recent developments

in the NMR spectroscopy namely the use of 100 Megac/cle and 220 Mega cycle instruments, computer averaged transient (CAT), double resonance and deuterium lebelling[52] have increased its potentiality to a great extent in getting an insight into the finer details of the molecular structure.

Evereince Shoolery and Rogers[53] had reported that the chemical shift of the angular methyl groups are dependent on the pattern of substitution in the steroid skeleton, the principle of additivity for signal shifts of angular methyl groups due to the shielding or deshielding effects of various nuclear substituents has been well established and documented[54-60]. Recently Uhram et al.[61] have attempted the calculation of the displacement effect of the side chain substituents on the resonance positions of angular methyl groups, which are given in table 2 . The rule of additivity of shift values of angular methyl groups has also been found to be valid in case of bufadienolides[62]. It has been observed, however, that this additivity no longer holds good when an alteration in the relative position of a relevant functional group and an angular methyl group is caused by an interaction of this group with another group or by conformational change of a steroidal rings[63-68]. The shift value of the angular methyl group is considerably larger when a substituent is introduced into a position 1,3, diaxial to the methyl group, than when introduced into other

Table 2 : Effect of side chain substituent on the chemical shift of C_{18} and C_{19}-methyl resonance.

Functional groups or 5,7 steroid nucleous		C-19 H H_z	C-18 H H_z
17β		-1.5	-2.0
17β		-2.5	-2.5
17β		0	0
17β		0	0
17β		-1.0	-1.5
17β		-1.0	-1.5

positions [54-60, 63,68, 69-71]. It has also been observed that because of symmetry, the same change in the substituents at analogous positions produce the same shift in the signal positions of an angular methyl group[54-60,72].

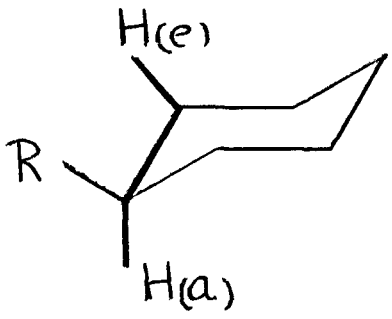
Differences in the chemical shifts[53] and the splitting pattern of the signals[73] of axial and equatorial protons attached to a substituent bearing carbon atom have been used to establish the configuration of that substituent. In general an axial proton in the cyclohexane ring resonates at a higher field than the equatorial proton[74]. The spin coupling constants of the signals due to protons attached to the substituents bearing carbon atoms have also revealed the flexible boat conformations of ring A[63-68,75,76] or distorted conformation of other rings[69-71,77]. The effect of various substituents upon the chemical shift of the protons attached to other carbon atoms of ring A have been studied. The shift values of a proton signal due to substituents vary depending upon their spatial relations. Most of the 6-substituents only slightly affect the 3α-proton signal. The effect of more proximate substituents would be more. Some polar 5α-substituents are reported to effect large deshielding of the 3α-protons[63-68].

McGarrey[55] has pointed out that a 6β-methyl group lowers the 19-methyl resonance by 2 to 4 Hz while 6α-methyl

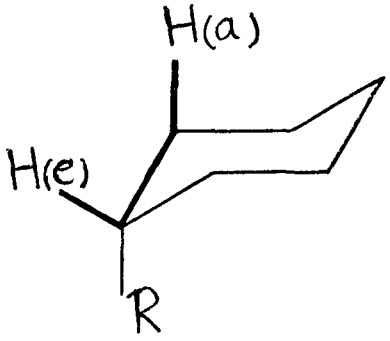
shows little or no effect. Moreover the resonances (doublets) for 6β-methyl were generally lower than the 6α-methyl group, probably due to 1,3-diaxial interaction of the former with the 19-methyl group. This observation is helpful in determining the configuration of 6-methyl groups.

It has been possible to distinguish between the hydrocarbon side chains of the steroids, namely cholestane, ergostane and sitostane type from one another by NMR analysis of relevant compounds[78]. The stereochemistry of the side chain of steroidal sapogenins have also been studied by NMR[79]. Richards et al.[80] have studied the NMR of 11-keto steroids. They have obtained the chemical shifts of the substituent groups together with constitutive values for their displacing effect upon the resonance positions of angular methyl groups. The 12-methylene proton signals of 11-keto steroids are characteristically affected by various substituents in ring C and D, which can, therefore, be easily located[81].

Shacca and Williams[82] have reported a finding of considerable importance that the coupling constant of axial and equatorial protons has the normal value (5.5±1.0 Hz) in the system(II), R = OH or OAc, but in(III), it is much reduced (2.5-3.2 Hz) for the proton sharing the substituted carbon atom. In the systems such as(II)and(III)the half width of the band due to equatorial proton is 5-10 Hz, and for axial proton it is 15-30 Hz[83].



(II)



(III)

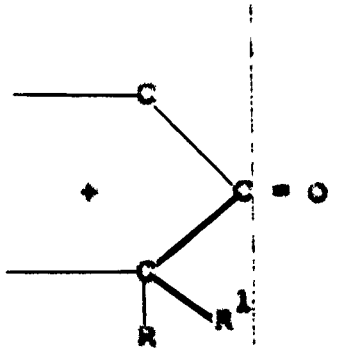
Solvent Effects: The proton resonances in NMR are much dependent on the nature of the solvent used. Hence, by obtaining the spectrum of the same substance in two or more solvents the ratios $\Delta\nu/J$ for some of the signals may change. The spectrum may, therefore, become more amenable to analysis. This procedure is sometimes a convenient alternative to obtaining the spectra at two different fields. The solvent shifts arise principally from the formation of collision complexes between the solvent and the solute. A collision complex, in general, will have a definite shape and various protons of the solute molecule will bear different spatial relationships to the complexed solvent molecule and will experience different magnetic environments due to its long range effects. Thus the solvent shift will give an information about the relative positions of the affected protons, and the stereochemistry of the solute. For getting the desired information from the solvent shift,

the NMR spectra of a compound is recorded in two solvents one of which must be inert and the other must be capable of interacting strongly with the solute. The active solvent must not only be capable of bonding with solute molecule at a particular site but must also have strong long range, directed effect on the chemical shifts of protons lying in its vicinity. Benzene and pyridine satisfy these requisites and are suitable for this purpose. The most widely used solvent pairs in the investigation of steroids are $CDCl_3$-C_6H_6 and $CDCl_3$-pyridine. The use of polar solvents such as dimethylsulfoxide[34] and dimethyl formamide[35] in conjunction with pyridine have been investigated for a large number of steroids. These combinations are helpful when the solubility limitations preclude the use of CCl_4 or $CDCl_3$.

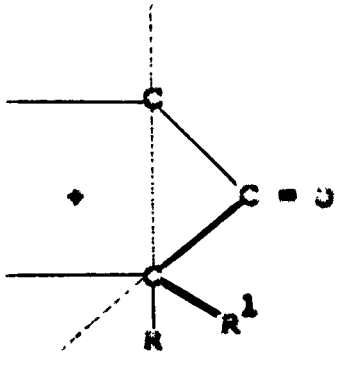
For the formation of collision complexes, the solute must carry certain sites for bonding with the solvents such π-electrons or polar groups capable of hydrogen bonding. Thus in case of keto compounds the π-electrons of the benzene ring interact with the partial positive charge on the carbonyl carbon atom in such a manner that π-electrons are as far as possible from the partial negative charge on oxygen. Previously, the association of benzene and C-O dipole was considered to be the association of benzene and C-O dipole was considered to be approximately planar[86-88]. Later, this planar association of benzene with the solute

was found to be untenable on the ground that the calculation for the magnetic field around a benzene ring can not account for the deshielding of the equatorial protons adjacent to carbonyl with shielding of the corresponding axial protons. Further, it was shown that the steric factors which would prevent even the approximately planar association, do not drastically change the solvent shift[89]. The concept of planar association has, therefore, been given up. A ketone benzene collision complex in which the plane of the benzene ring is almost at right angles to the overall plane of the steroid molecule, accounts well for the observed shifts[90].

As a rule benzene (relative to $CDCl_3$) causes the shielding of the protons lying behind a plane down through carbonyl carbon perpendicular to the direction of the carbonyl bond. The protons lying in front of the plane are deshielded. Pyridine, however, tends to shield the protons lying behind a plane passing through the α-carbon atoms and deshields those lying in front of this plane as shown in figs. (IV) and (V).



(IV)



(V)

The solvent shift ($\triangle = \delta CDCl_3 - \delta C_6H_6$) associated with carbonyl groups frequently remain dominent even in the presence of other functionalities and are approximately additive. Solvent shifts are, therefore, useful in establishing the spatial relationship between ketones and protons whose resonances can be discerned in both solvents. Proximity of a highly polar substituent can change the solvent shift which is characteristic of an isolated carbonyl group. Solvent shifts in the diketones are approximately additive. Introduction of one or two equatorial halogen atoms adjacent to the keto group increase the shift value of C_{19} methyl protons in 3-keto steroids[91].

In the correlative studies of the solvent shifts with the structure of some hydroxy and methoxy steroids Williams et al.[92] have observed that the protons attached to the carbon bearing the oxygen function exhibited positive shift values of the order of 0.05-0.25 ppm. in benzene. Protons in proximity of the lone pair of electrons of an oxygen atom showed negative solvent shift. Protons of the angular methyl groups bearing a 1:3 diaxial or quasidiaxial relationship to the hydroxyl group showed negative values. The pyridine induced shift in the NMR spectra of hydroxylic compounds have also been investigated[93]. In the case of saturated steroid hydrocarbons the magnitude and sign of the benzene induced resonance shifts of methyl and methylene protons due

to nonpolar effect have been demonstrated to depend upon the shape of the molecule. In the polar solutes the solvents shifts have been shown to originate from the polar as well as non-polar effects. Based on the solvent induced shift of the 19-methyl groups in 4β, 6β epoxy-1-OXO-steroids, ring A nas been shown to possess a boat conformation whereas similar studies on a 4β -acetoxy-5β-hydroxy-1-oxo-steroids showed a chair conformation for ring A[94].

Bhacca and Williams have studied the correlation between the solvent shifts of angular methyl groups and skeletal substituents in several steroidal sapogenins and some steroids ketones and acetates[36,95]. The solvent shifts of the angular methyl signals have been widely used in establishing the position of functional groups and stereochemistry of a wide range of natural steroids[43,96-113].

Shift-Reagents: The most recent addition to the armoury of NMR spectroscopy is the use of paramagnetic shift reagents (PSR). The use of these reagents has greatly enhanced the power and versatility of NMR spectroscopy and has provided a new method for locating the functional groups in the organic compounds. In fact they have opened up a new field of study ever since the discovery by C.C. Hinckley[114] that proton resonance signals of cholesterol were dramatically shifted downfield in presence of Eu $(thd)_3$ giving a very

simplified spectrum. Paramagnetic shift reagents are complexes of lanthanide ions with organic ligands which, when added to the solution of electron lone pair bearing organic compounds, induce low field shifts of the order of 0-20 ppm. in the proton resonances, leading to the striking spectral simplifications. Most widely used PSR are Tris(2,2,6,6-tetramethyl-heptane-3,5-dianato)praseodymium, $Pr(tmhd)_3$[115] and Tris (dipiralomethanato)europium $Eu(dpm)_3$[116]. Presently $Eu(dpm)_3$ is considered to be the best PSR. The PSR forms a complex with the substrate at OH or other groups bearing lone pair of electrons. The low field shifts produced by the reagents are approximately proportional to r^{-3}(r = Eu-H distance).

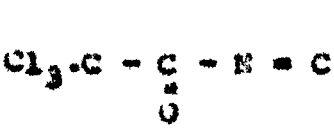
The paramagnetic shift is predominantly caused by a pseudocontact interaction, although the possibility of small contribution of contact interaction, (particularly when π-systems are available) can not be ruled out. Pseudocontact shift arises only through the magnetic dipolar field effect of unpaired electrons and does not affect the bond electron density and hence also the coupling constant[117]. The magnitude of the complexation of the PSR with the functional groups varies in order $NH_2 > OH > CO > -O- > COOR > CN$[118]. The substitution pattern of upto six to seven carbon atoms removed from the site of co-ordination is frequently revealed in the cyclic systems. Thus the location of the functional

groups in a known carbon skeleton can be determined by measuring the relative shift ratios, e.g., of 18 and 19-methyl groups in a steroid. However, such analysis is possible only with monofunctional molecules. Polyfunctional compounds tend to complex at more than one site leading to a confusing spectrum. These difficulties can be surmounted by suitably blocking the interfering functional groups, e.g., a ketone may be converted to a thioketal and hydroxy group to a trifluoro-acetate[119]. Reports of the use of PSR in the steroidal field are frequently encountered in the literature[120] and hold great promise for future studies.

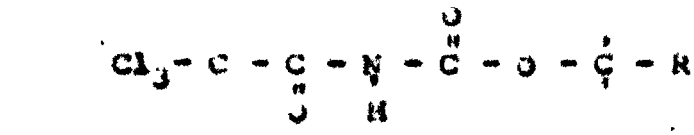
Reagents for Classification of Alcohols : Use of various reagents in the NMR spectroscopy for determining the nature, substitution position and configuration of hydroxyl groups in steroids has been reported during last few years. King[121] has described the use of DMSO for classification of alcohols. In DMSO solution the hydroxyl resonance is shifted downfield (τ6 or lower) due to strong hydrogen bonding and the splitting of hydroxyl proton can also be observed because of much reduced rate of proton exchange. The primary, secondary and tertiary alcohols display clearly resolved triplets, doublets and singlets respectively. Polyhydroxy compounds give separate peaks for each hydroxyl proton. The equatorial hydroxyl proton resonates at lower field than the axial hydroxyl proton. Hemiacetal and hemiketal protons appear as

doublets and singlets respectively, at lower field than the ordinary hydroxyl proton.

Trichloroacetyl isocyanate (TAI) (VI) has been used for *in situ* reaction with alcohols for NMR studies[122]. TAI reacts instantaneously even with the highly hindered hydroxyl groups such as a tertiary OH and 11β-OH, to form a carbamate (VII). The carbamate N-H signals usually appear in the region



(VI)



(VII)

of τ 1-2 as distinct singlets, the number of which is a measure of the total number of hydroxyl functions. The carbamate formation shifts the carbinol protons downfield by 0.5 to 0.9 ppm. for primary alcohols and 1-1.5 ppm. for secondary alcohols. In case of allylic alcohols, the vinyl proton β to the hydroxyl group shifts downfield by about 10 to 15 cps. on carbamate formation whereas the α-vinyl proton suffers only a little shift.

Like TAI, chloral (trichloro-acetaldehyde) also reacts rapidly at room temperature with certain hydroxy steroids, leading to the formation of two isomeric chloralates with R and S configuration in varying ratios. The NMR of the reaction mixture displays sharp singlet in the region of

τ4.7 to 5.2 due to the tertiary protons of the R and S chloralates. The difference in the chemical shift ($\triangle$) of these singlets and their τ values are found to be fairly characteristic of the substitution position of the hydroxyl groups in the sterols[123].

^{13}C - NMR Spectroscopy : Although proton NMR have been widely used in structural and conformational studies, ^{13}C-NMR spectra of steroids are being found to be particularly useful because of the high sensitivity of ^{13}C chemical shifts to structural changes and also because of the ease with which each carbon atom of the skeleton and the substituents groups can be examined individually.

J.D. Roberts et al.[124] have studied the ^{13}C resonance spectra of a variety of sterols and for most of the steroids it was possible to resolve all the carbon resonances from one another with aid of complete proton decoupling. The unambiguous assignments of nearly all the resonances have been made by the application of specific single frequency and off-resonance proton decoupling and by hydroxyl acetylation effects on chemical shifts using deuterium labelled steroids. ^{13}C-NMR spectroscopy has been used to identify the steroidal components of Green River shale bitumen[125].

Mass spectrometry :

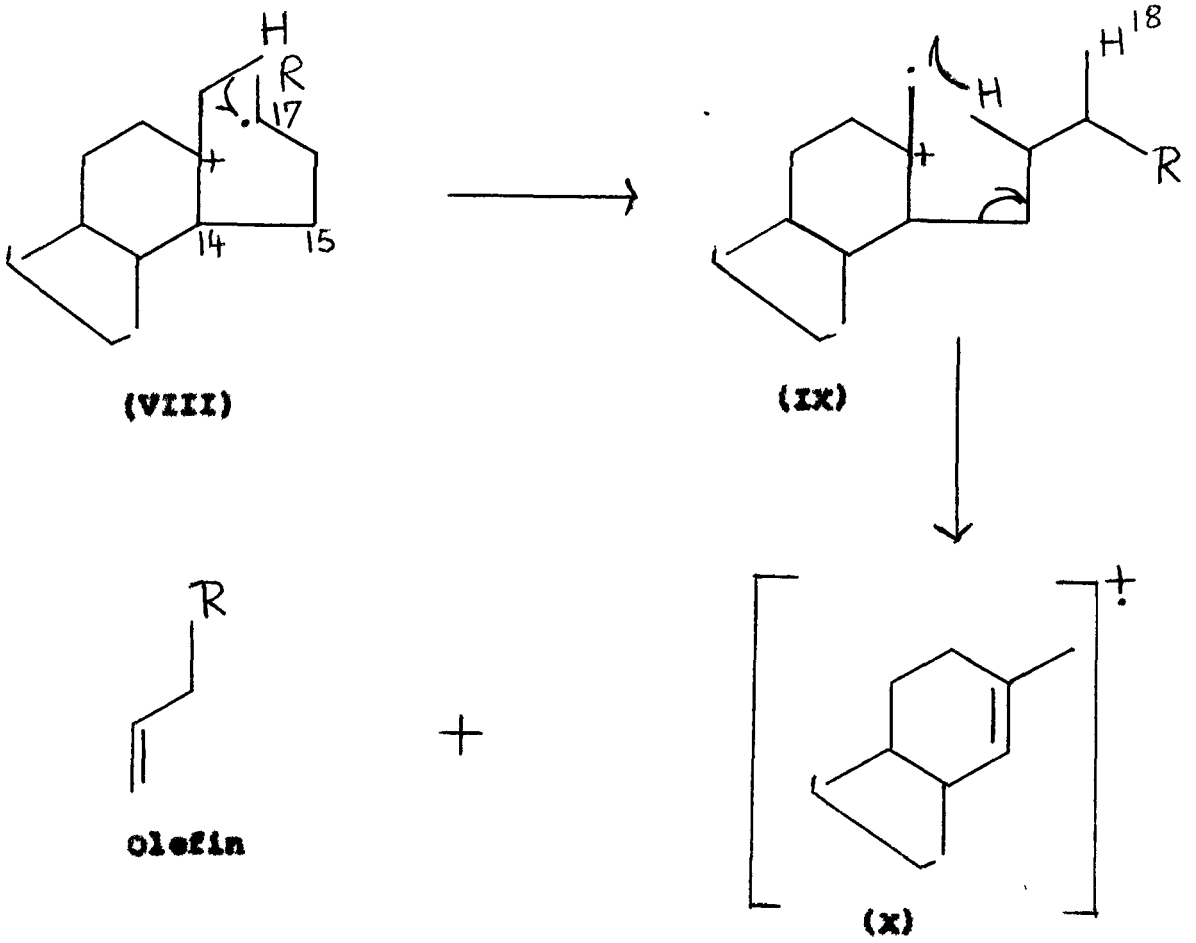
The mass spectrometry of steroids has seen major advancements only during the last few years, chiefly because of the incorporation of suitable inlet systems in the mass instruments, development of computerised high resolution mass-spectrometer and combination of GLC apparatus with the mass-spectrometer. The characterization of steroids by gas-liquid chromatogram-mass spectrometer is of special significance in dealing with microquantities of steroid material[126-131] from biological sources. Recent development of GLC columns suitable for deuteration of steroids during chromatography have considerably enhanced the potentiality of this combination[34].

Djerassi and his school have established themselves as pioneers in the field of steroid mass spectrometry, having unravelled the mechanism of most modes of the fragmentation of steroids under electron impact. The mechanism of mass fragmentation is generally postulated by studying the mass spectra of compounds labelled by deuterium at suitable positions. In all the mass spectra of steroids, certain fragmentation processes are inherent in the steroidal skeleton. The presence of a functional group in the skeleton may, however, trigger off certain other fragmentations, which are characteristic of the functional group in a specific position. The location of the functional groups may, therefore,

be narrowed down by consideration of the cracking pattern. Further, the functional groups may be suitably modified by derivatisation, oxidation and reduction etc. to induce characteristic patterns in the mass-spectrum, e.g., an alcohol may be oxidised to ketone[132] which in turn, may be converted to a ketal. The general modes of fragmentation of steroids have been dealt with in detail by Biemann[133] and Budzikiewicz et al.[134]

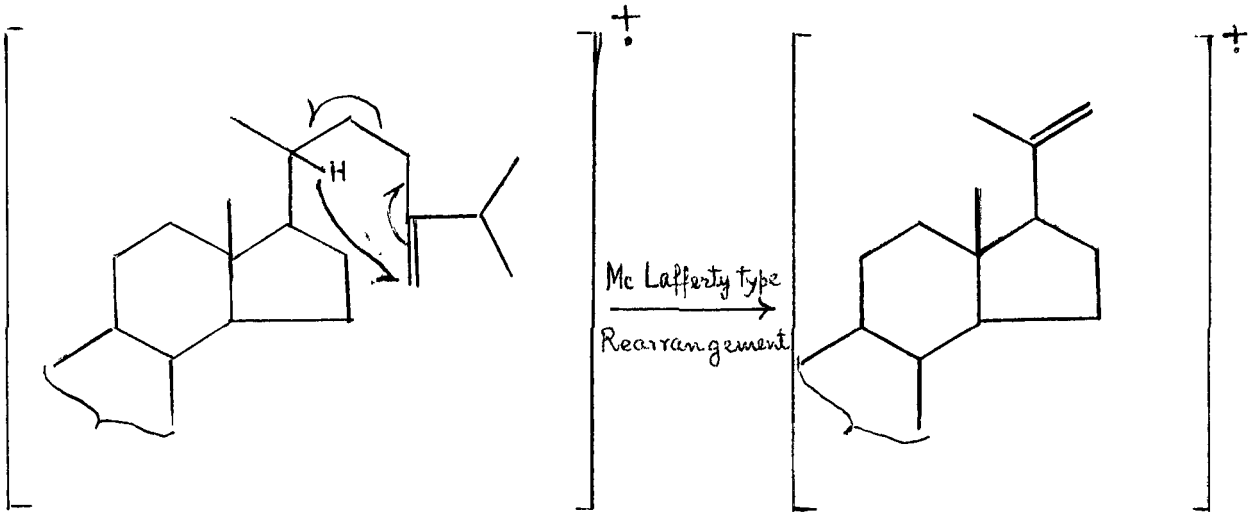
The facile elimination of the side chain +42 mass units is a common feature of steroid skeleton and is of considerable utility in infering the size and nature of the side chain in the steroids. Several mechanistic proposals have been made about the nature of this fragmentation process. Only recently Djerassi[135] has shown by extensive deuterium labelling experiments that the first step in this fragmentation is the formation of the molecular ions(VIII), by the rupture of highly substituted 13-17 bond releasing the strain inherent to the trans-hydrindan system. The transfer of a hydrogen atom from C_{18} which is activated by the adjacent positive charge, relieves the radical site on C_{17} yielding an ionised olefin(IX). A hydrogen atom is then abstracted by the C_{18} radical from C_{16} in a six-membered transition

state followed by the cleavage of 14-15 bond, yielding ion (X) with the loss of a neutral olefin (side chain +42).

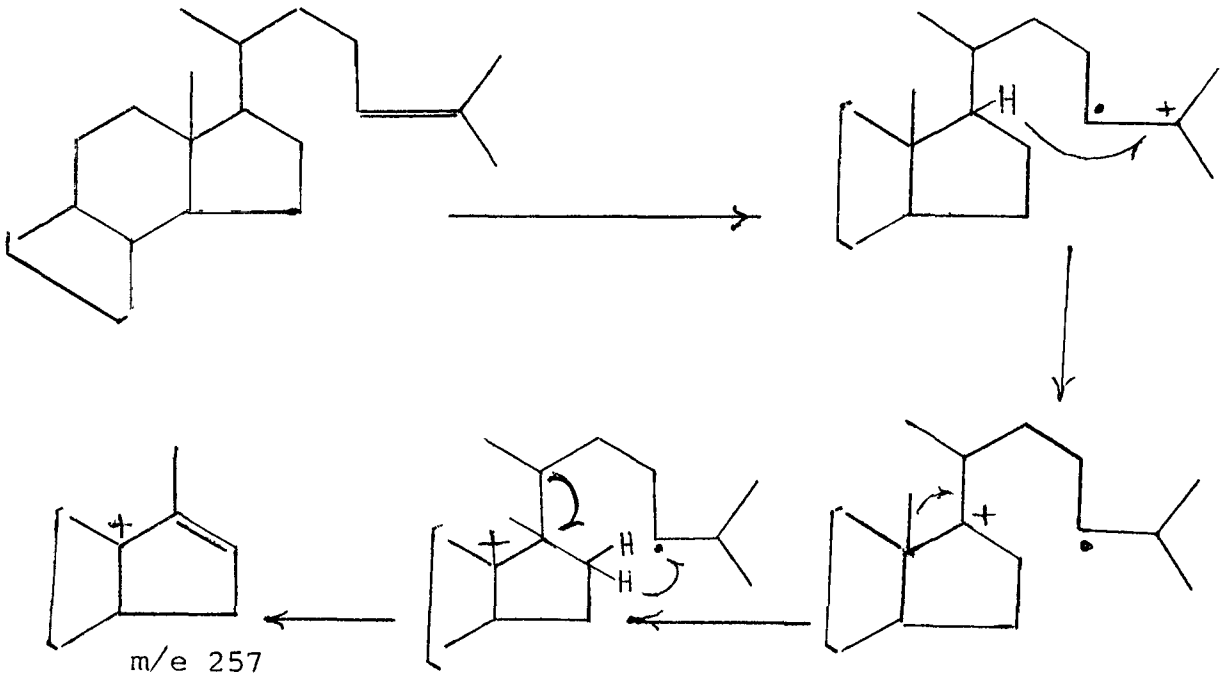


Presence of certain functional groups in the skeleton may, however, supress this normal cleavage of ring D. Thus, the presence of a hydroxyl group at C-12 leads to the elimination of side chain +18 mass units instead of side chain +42 mass units[136]. Further, a double bond in the side chain at certain positions also precludes this elimination which is not surprising since the preferred charge localisation at the

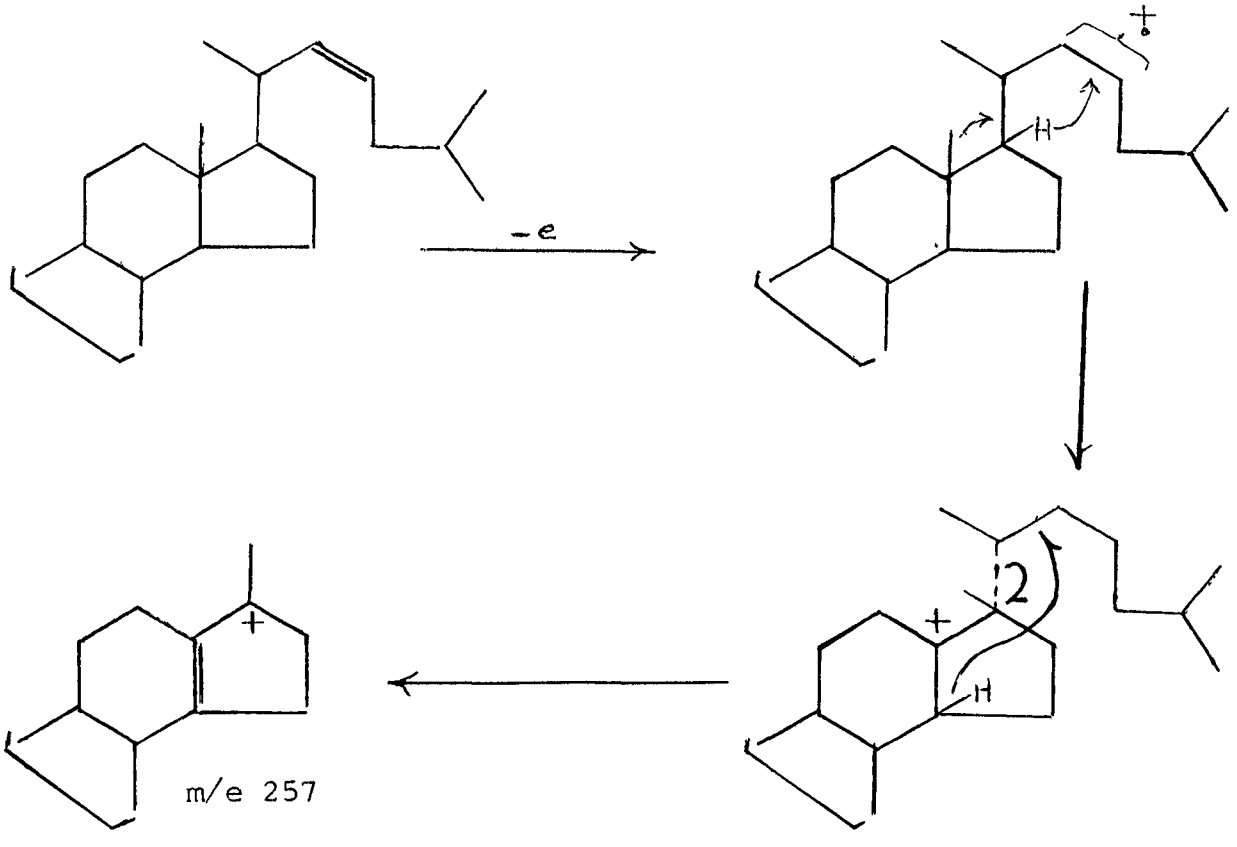
double bond is to be anticipated at the expense of contributions from the species(VIII). The mass fragmentations of several sterols with unsaturated side chains have been studied[137]. One of the most diagnostic fragmentation of such sterols involve the loss of entire C_{17} substituent together with two skeletal hydrogen atoms. However, the mechanism of this elimination differs, according to the position of unsaturation in the side chain and has been classified through the use of deuterium labelled steroids, as given in the schemes 1-3.



Scheme 1



Scheme 2

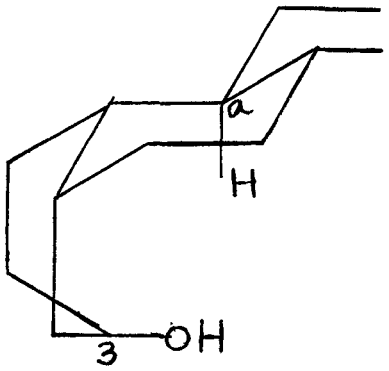


Scheme 3

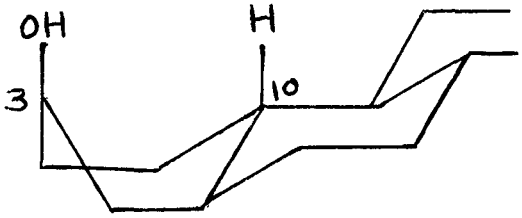
The skeletal double bond whenever present or generated by the elimination of water or acetic acid from the molecule sometimes leads to Retro-Diels Alder fragmentation permitting to localise its position. The steroidal Δ^4 and Δ^5-3-ketals can readily be identified by the presence of diagnostic peaks at M-28 and m/e 99 mass units respectively in their mass spectra[138].

Smith and co-workers[139] have reported an elegant method of distinguishing between 19-methyl and 19-nor-steroids with the help of high resolution mass spectrometry. The 3-oxo derivative of the steroid is converted to the 2-spiro-2'-(1,3-dithiane) and the ratio of the peaks in the mass spectrum at m/e 145 and m/e 159 due to ions $C_6H_9S_2^+$ and $C_7H_{11}S_2^+$ respectively are determined. This ratio has been found to be 1:20 for 19-methyl steroids but reversed for 19-nor compounds. These results were not reliable with the steroids having a double bond in ring A or B. The configuration of steroid alcohols can easily be established from the intensity ratio of the $M-H_2O$ peak to the M^+ peaks in their mass spectra. For an axial hydroxyl group the ratio $M-H_2O/M^+$ is more than one whereas for equatorial hydroxyl group it is less than one[140]. In case of 3-hydroxy steroids the loss of H_2O is more facile in the 5βH than in 5αH series. This has been rationalised by assuming that the elimination of H_2O involves the participation of 9αH in a boat form (XI). In case of 19-nor

steroids, H_2O is easily lost if the hydroxy group occupies a position cis to the 10β H in a boat form (XII). Presence of one or two methyl groups at C_1 in 3-hydroxy-5α-H steroids facilitates the dehydration by involving a hydrogen atom on CH_3 group in the 1,4 elimination. A Δ^5 double bond also brings about easier cleavage of H_2O[141]. More recently



(XI)



(XII)

Djerassi[142] has classified the cause of common and structurally significant fragmentations of steroids by elucidating the nature of 3 types of hydrogen rearrangements (single H-transfer, double reciprocal H-transfer, and double unidirectional H-transfer) by extensive deuterium labelling.

The development of molecular mass spectrography by Ardenne et al.[143] is another step forward in this field, wherein negatively charged M-1 ions are formed by the addition of low energy electrons to the molecules followed by elimination of one H, usually with fragmentation. Other peaks encountered at secondary masses arise from the thermal

elimination of water and acetic acid from the hydroxy and acetyloxy steroids respectively at the temperature of volatilization. Sometimes the addition of an O^- ion to the molecule results in a M-1+16 peak. This technique allows an accurate determination of molecular weight upto 2000. It also enables the determination of molecular weight distribution in the complex mixture of similar steroids. In the structure elucidation studies of cardenolide genins and glycosides[144-146] and other steroids,[147] this method is increasingly being resorted to.

Optical Rotatory Dispersion and Circular Dichroism :

The phenomenon of circular dichroism (C.D.) and optical rotatory dispersion (O.R.D.) are closely related. The CD results from the unequal absorption of right and left circularly polarised light by the optically active medium, while ORD refers to the change in optical rotation with wave length. The wave length regions corresponding to the absorption bands of the chromophore in question are of special interest, and for most of the colourless organic compounds, this corresponds to the ultraviolet range[148,149].

Eversince their development, both these techniques have mainly been used for the investigation of carbonyl group in rigid asymmetric structures such as steroids and polycyclic terpenes, because the keto group undergoes a weak

optical transition in the vicinity of 280 to 300 nm region. This transition is fairly sensitive to the induced asymmetry and is readily available to serve as an ideal probe for revealing the finer structural, configurational and conformational details of a particular molecular frame work.

For locating a carbonyl group in a steroid skeleton ORD curves have generally been found to be more useful than CD curves, because the former exhibit different shapes as well as signs which are absent in CD. It has, however, been noted that when the CD curves are recorded at ca -192^{0} vibrational fine structures usually develop in the curve which are frequently characteristic of a carbonyl group in a given environment and thus may be employed for locating it[150,151]. Steroidal ketones exhibit a strong cotton effect between 185 to 195 nm. The α and β axial methyl and methylene groups make large contributions and their signs are determined by the Octant Rule[152].

The double bond present in the asymmetric cyclic structures exhibit a cotton effect at ca 200 nm. The amplitude and sign of the effect are constant for each position of the double bond in the skeleton but they vary when the immediate enviorenment of the chromophore is modified. A double bond in a steroid skeleton can, therefore, easily be characterised by CD[153,154].

The strong cotton effect of benzoate resulting from $\pi \rightarrow \pi^*$ intramolecular charge transfer transition at ca 225 nm. has been used to predict the absolute configurations of secondary hydroxyl groups in steroids[155].

The CD of a series of etianic acids have been studied at low temperature (-10 to -18°) and the increase in the dichroic absorption ($\Delta\varepsilon$) with the decreasing temperature was found to be linear. This has been attributed to an increase in the proportion of molecules adopting the preferred conformation at the lower temperature[156]. Further, CD and ORD have also been used to follow the formation of ketals from optically active ketones, since the ketal formation leads to a proportional decrease of the cotton effect[157].

Compounds containing hydroxy groups as the only substituent on a saturated steroid skeleton show no absorption above 200 nm. but show well defined cotton effect in the region 185 to 198 nm. It is, therefore, possible from their CD studies to predict the preferred configuration of the hydroxy groups. Moreover, the hydroxyl cotton effect can no longer be ignored in the CD studies of other chromophores such as olefins and ketones (short wave length band) which absorb below 200 nm.[158]

Takemoto et al.[100] have confirmed the presence of 5β-OH group in sengosterone by comparison of ORD and CD

curves ($n \rightarrow \pi^*$ transition of CO group) of sengosterone with those of cyasteMone which has a 5β-hydrogen. The CD and ORD have become indispensable in tackling stereochemical problems pertaining to steroids, their use in the steroids on, e.g., insect moulting hormones are well documented[99,100,106-110].

X-RAY Crystallography :

X-ray crystallographic analysis is the last resort in the elucidation of absolute stereochemical structure of organic compounds. In the field of steroids, this technique has been used for structure determination of a large number of compounds[2]. A few recent examples of its application, in this field, are the structure elucidation of carpesterol[159], physalin A[160], physalin B[161] and physalin C[162].

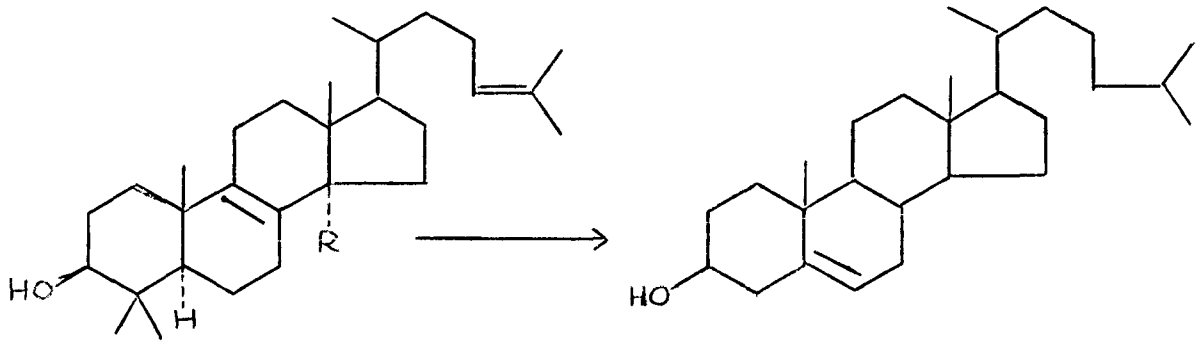
Biosynthesis :

All the steroids are biogenetically derivable from the same parent compound, isoprene (C_5H_8). Biogenetic experiments using tracer techniques in a great variety of organisms and systems have shown that the biosynthesis of sterols proceeds through a sequence :

Mevalonate ⟶ Farnesol ⟶ Squalene ⟶ Lanosterol

These steps are in general, well reviewed[163-165]. The most interesting aspect of steroid biosynthesis involves, the

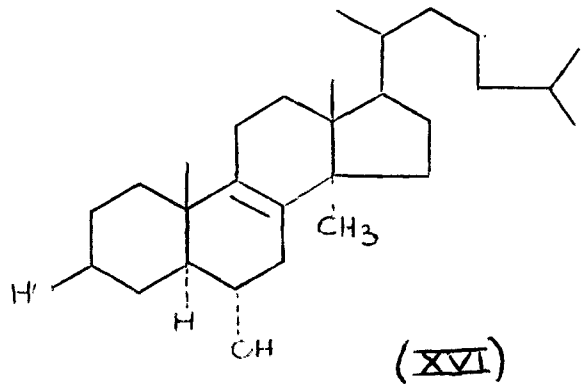
terminal stages in the conversion of lanosterol (XIII) to cholesterol (XV), namely the demethylation[166] of lanosterol, which proceeds through the initial elimination of 14α-methyl group followed by the oxidative removal of the two methyl groups at C_4. The evidence in support of this path was obtained by Bloch[167-169] who demonstrated the intermediacy of 14-norlanosterol (XIV)[170-173] in cholesterol biosynthesis in rats. The isolation of 4-monomethyl sterols, e.g., lophenol[170] from animal as well as plant sources also substantiates this proposition. Djerassi et al.[174] have isolated a 14-monomethyl sterol, macdengallin (XVI) from certain cacti and inferred that at least in the cactus, the demethylation in ring A can precede the removal of 14-methyl group of a lanosterol-like precursor. The origin of ethyl and ethylidene groups at C_{24} of the side chain, characteristic of phytosterols is now well established. These are derived by double transmethylation from adenosylmethionine[175-177]. The first transfer of a methyl group to a $\Delta^{24(25)}$ double bond leads to C_{24}-methylene compound(XX)with migration of a hydrogen atom from C_{24} to C_{25}[178,179]. The second transmethylation at the $\Delta^{24(28)}$ double bond of compound(XX)leads to the carbonium ion(XXI). The latter can be stabilised by several routes (a, b, c and d)[180].



(XIII), R = CH_3

(XIV), R = H

(XV)

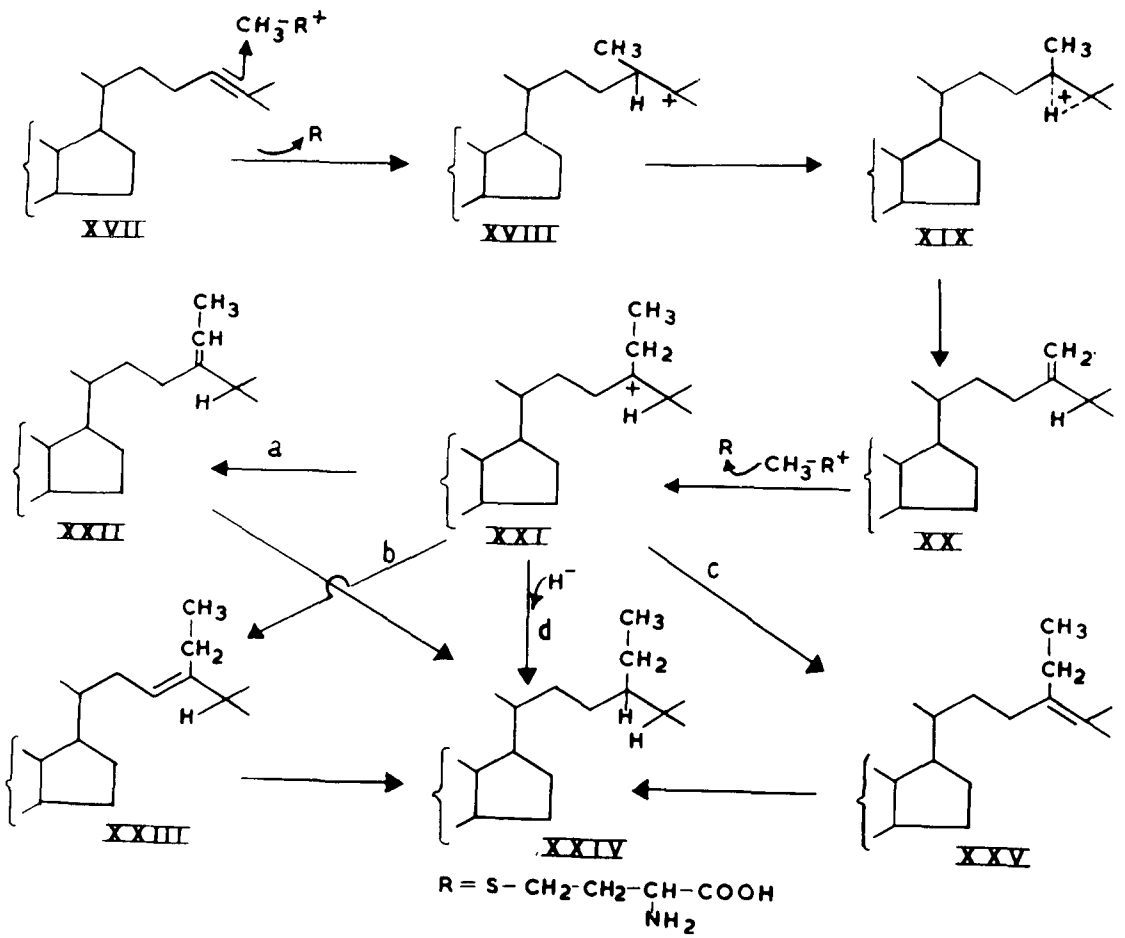


(XVI)

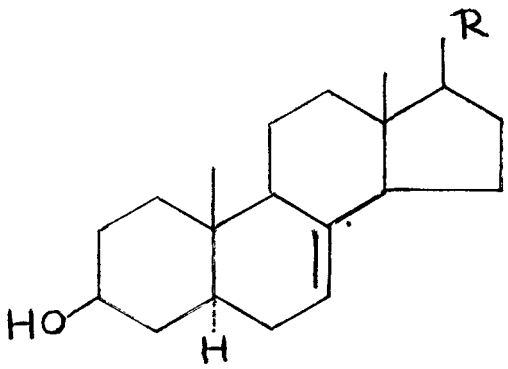
In case of poriferasterol from Ochromonas malhamensis[181], the 24-ethylidene compound (XXII) has been proved to be the precursor. On the other hand, Leufant et al.[182] have shown that in Dictyostelium discoideum stigmastene-22-en-3β-ol is formed without the intermediacy of a C_{24}-ethylidene compound. This intermediate is also not encountered in the biosynthesis of chondsillasterol, Δ^7-chondsillasterol and poriferasterol in Chlorella[183,184].

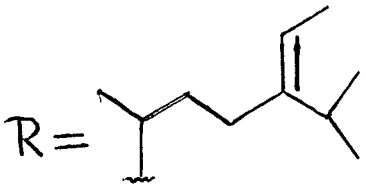
In the biosynthesis of 24-ethylcholesta-5,22,25 trien-3β-ol from Clerodendrum speciosum path 'd' of scheme-3 is reported to be operative[185].

SCHEME 3

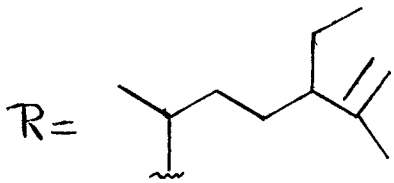

CH3-R+
R
CH3
H
+
XVII
XVIII
XIX
CH
XXII
CH2
XXI
XX
a
b
c
d
H-
XXIII
XXIV
XXV
R = S- CH2-CH2-CH-COOH
NH2

Raduchel and Sucrow[186] isolated compounds (XXVI), (XXVII) and (XXVIII) from the pumpkin plants fed with compound (XXV). It was, therefore, concluded that compound (XXV) was the precursor of the isolated products. The earlier

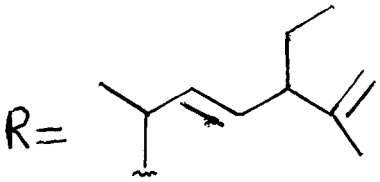




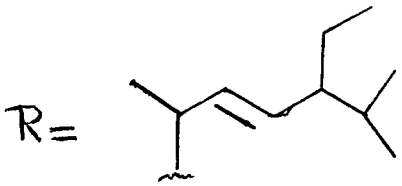
(XXV)



(XXVII)



(XXVI)



(XXVIII)

proposition[187] that Δ^{25} and $\Delta^{24(28)}$ sterols are formed from the common intermediate is untenable in case of pumpkin plants, since the $\Delta^{24(28)}$ compound (XXV) is first converted into a C_{24} carbonium-ion by addition of a proton, a hydride transfer

from C_{25} to C_{24} then results in a C_{25} carbonium-ion, which by proton loss from C_{26} leads to a Δ^{25} double bond. Whether compound (XXVII) is formed through compound (XXVI) or directly from compound (XXV) is yet to be established. Only a small incorporation of compound (XXVIII) (∝-spinasterol) indicates that it is not generated directly from (XXV).

Two probable routes for the biosynthesis of stigmasta-22-en-3β-ol in Dictyostelium discoideum have been proposed[137]. It is formed either by elimination of H-atoms at C_{22} and C_{23} of stigmastanol or it is formed during ethyl branching at C_{24} of lanosterol by elimination of the hydrogen at C_{22} accompanied by migration of the C_{23} hydrogen to C_{24}.

Although not much work is reported on the biosynthesis of insect moulting hormones in plants but for the formation of ecdysterone in the seeding of Podocarpus elata, it has been established that cholesterol is the precursor[138].

Microbiological transformations of Steroids :

It is known for a long time that microorganisms possess enzymes capable of transforming the steroid molecule. Interest in these transformations arose particularly when Peterson and Murray in 1952 succeeded in converting progesterone into 11∝-hydroxyprogesterone by a fungus of Rhizopus genus. The selective introduction of an oxygen function at 11-position was considered of much interest in

the partial or total synthesis of adreno-cortical hormones, since the oxygenation at this position by purely chemical means proved to be rather unrewarding. Intensive investigations in this field were therefore, undertaken. Although the steroid transforming ability is widespread in the realm of microorganisms, filamentus fungi have been found to be the most versatile.

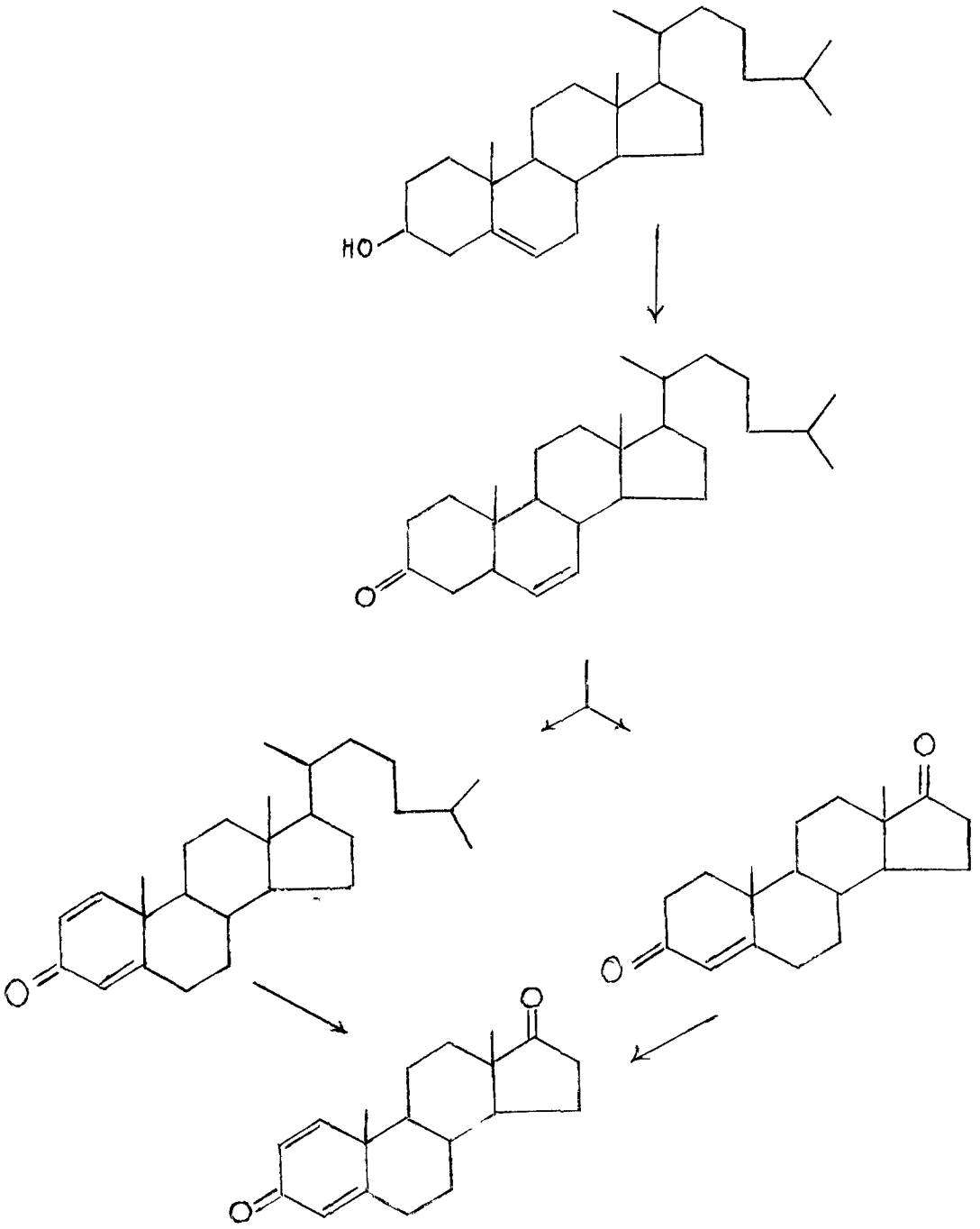
Various microbial transformations of the steroids can be classified as oxygenations, (e.g. hydroxylation, epoxidation, lactone and ketone formation), hydrogenation, dehydrogenation and cleavage of the side chain. These steroid transformations by usual microbial methods have been thoroughly reviewed by Vischer and Wettstein[189].

Hydroxylation is the most frequently encountered reactions in the microbial transformations of steroids. The hydroxy group can be introduced almost at any position in the steroid nucleus[189,190]. In certain cases the site and efficiency of hydroxylation by a particular microorganism are strongly influenced by the structure of the substrate. Thus, the direct hydroxylation of the readily available androstane is not possible by Penicillium urticae, Bain (a known 15-hydroxylating organism). However, the substrate (viz. 5α-androsta-3,11-dione) suitable for this organism could be

prepared from androstane by first 11α-hydroxylation with Aspergillus Ochraceus, Wilhelm, followed by standard chemical reactions[191].

Acetylation of steroids in micro-biological systems has been reported only recently. Thus testosterone acetate has been obtained by the fermentation of androstenedione with a strain of Saccharomyces fragilis[192]. A Streptomyces spp has been used to reduce 3-keto-1,4-pregnadienes into 3-keto-1-pregnenes[193].

The painstaking researches of Arima et al.[194,195] have led to the selective cleavage of the side chains of various steroids namely cholesterol, 7-dehydrocholesterol, campesterol, β-sitosterol and stigmasterol by various microorganisms belonging to the genera, Arthrobacter, Bacillus, Brevibacterium, Corynebacterium, Microbacterium, Mycobacterium, Nocardia, Protaminobacter, Serratia and Streptomyces. The resultant product of the fermentation was androsta-1,4-diene,3,17-dione (A.D.D.). The following pathway exemplified by cholesterol has been found to be operative in the above cleavage.



Use of fungal spores (Conidia) instead of mycelia for steroid transformations has revealed interesting results. Spores of Aspergillus ochraceus have been found to be about five times more active than growing culture in transforming progesterone to 11-α-hydroxy and 6β, 11α-hydroxy

derivatives[196-199]. A large number of steroids are transformed into their 11α-hydroxy derivatives by spores of Aspergillus ochraceus. However, when acetylated substrates are used 11α-hydroxy derivatives of the corresponding alcohols are obtained. Spores of A. ochraceus bring about side chain cleavage along with 11α-hydroxylation of 16-dehydroprogesterone leading to the formation of 11α-hydroxy androsta-4-en-3,17-dione[199].

Geotrichum affinis spores have been used to bring about C-1 dehydrogenation, C-1 dehydrogenation with cleavage of C-17 side chain and aromatisation of ring A of 19-oil or 19-nor steroids. The side chain cleavage of pregnanes has been found to proceed in a way analogous to the non-enzymatic Baeyer-Villiger oxidation of ketones by peracids[200].

With the spores of Mucor griseocyanus deoxycorticosterone, testosterone, progesterone and androsta-4-en-3,17-dione can be transformed mainly to their 14α-hydroxy derivatives. However, 17α-alkyl derivatives yield 7α-hydroxy derivatives as the principal products[201].

Stigmastane Type of Natural Steroids :

During the course of structure elucidation of physanol A and physanol B from Physalis franchetti Mast. the author surveyed the literature on stigmastane type of

compounds and those characterised during the period under review have been classified into four groups. These are listed in tables 3 to 8 along with the plants of their origin, the plants in turn are arranged in an alphabetical order.

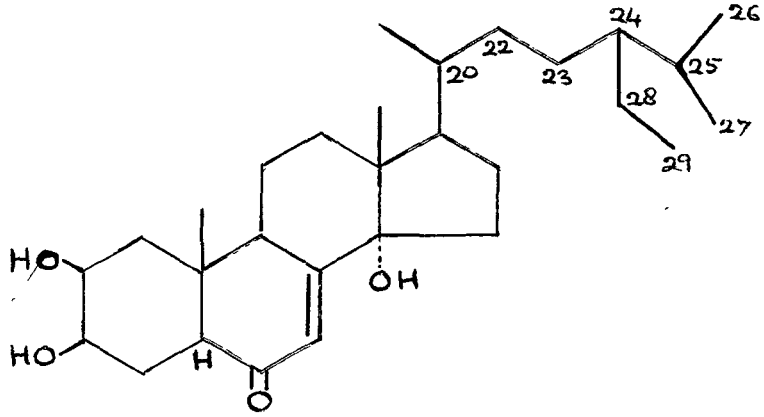


TABLE 3 : Insect moulting hormones without lactone groups.

Plant	Family	Name	Structure	Melting point °C	Rotation	U.V. max. mμ	Ref.
Ajuga incisa Maxim.	Labiatae	Ajugasterone-B	5βH, 20R, 22R, 25(26) Δ, 27-OH (24ξ).	240	54.7	244	110
Cyathula capitata Moquin-Tandon	Amaranthaceae	Amarasterone-A	5βH, 20R, 22R 26-OH, (24ξ)	210-211	-	244	109
Cyathula capitata Moquin-Tandon	Amaranthaceae	Amarasterone-B	5βH, 20R, 22R, 29-OH, (24ξ)	284-85	-	244	109

Contd.....

TABLE 3 : (Contd....)

Plant	Family	Name	Structure	Melting point $^{o}_{c}$	Rotation	U.V. max. mμ	Ref.
Podocarpus elatus R.Br.	Coniferae	Podecdysone-A*	5βH, 20R, 22R, 25-OH, (24ξ)	262-64	-	243	202
Podocarpus macrophyllus D.DON	Coniferae	Makisterone-C	5βH, 20ξ, 22ξ, 25-OH(24ξ)	263-65	-	243	108
Podocarpus macrophyllus D.DON	Coniferae	Makisterone-D	5βH, 20ξ, 22ξ, 28ξ-OH(24ξ)	non-crystalline	-	244	108

*Probable same as Lemmasterone m.p. 253-59° from Lemmaphyllum microphylum Presl.[111]

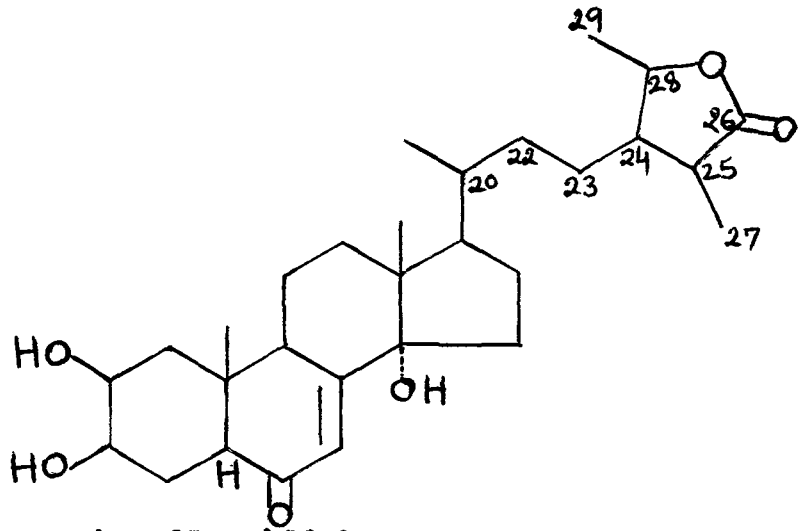


TABLE 4 : Insect moulting hormones with 28 ⟶ 26-lactone groups.

Plant	Family	Name	Structure	Melting point °C	Rotation	U.V. max. nm	Ref.
Cyathula capitata Moquin-Tandon	Amaranthaceae	Cyasterone	5β-H, 20ξ, 22ξ-OH, (24ξ, 25ξ, 28ξ)	164-66	+64.5	-	99
Cyathula capitata Moquin-Tandon	Amaranthaceae	Epicyasterone	5α-H, 20R, 22R-OH, (24S, 25R, 28R)	274-75	-	242	105
Cyathula capitata Moquin-Tandon	Amaranthaceae	Isocyasterone	5β-H, 20R, 22R-OH, (24S, 25S, 28R)	-	-	242	105
Cythula capitata Moquin-Tandon	Amaranthaceae	Sengosterone	5βξ, 20ξ, 22ξ-OH (24ξ, 28ξ)	159-61	+39.6	241	100

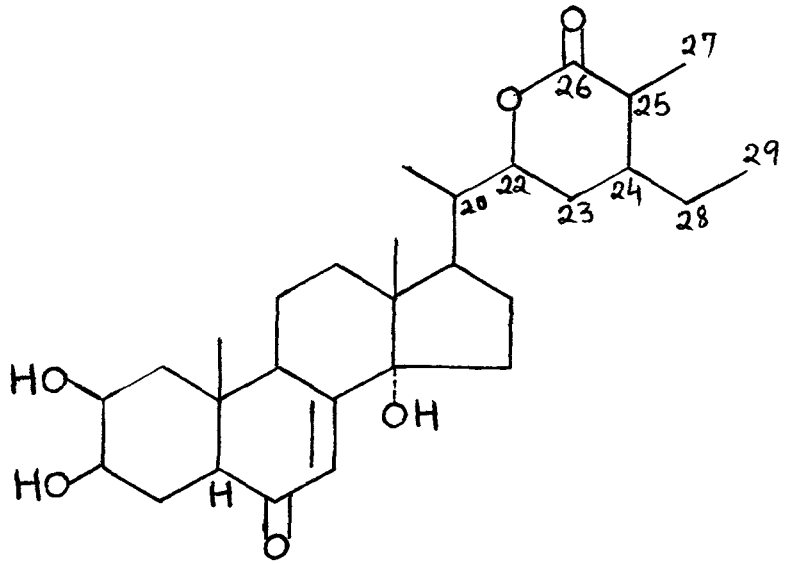


TABLE 5 : Insect moulting hormones with 22⟶26-lactone groups.

Plant	Family	Name	Structure	Melting point °C	Rotation	U.V. max. mμ	Ref.
Cyathula capitata Moquin-Tandon	Amaranthaceae	Capitasterone	5βH, 20ζ,-OH (22ζ, 24ζ)	234-35	-	242	107
Cyathula capitata Moquin-Tandon	Amaranthaceae	Precyasterone	5βH, 20ζ, 28ζ-OH, (22ζ, 24ζ)	-	39	244	106

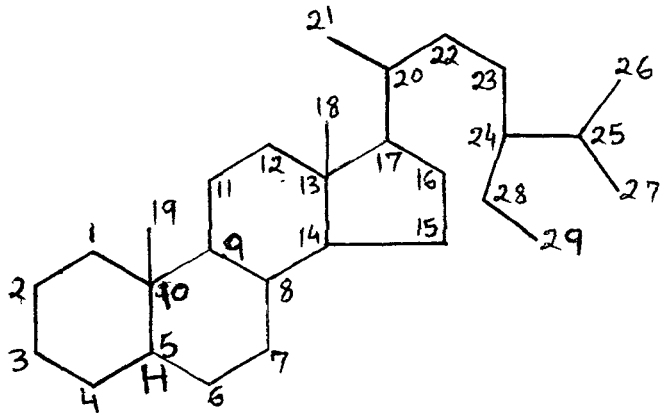


TABLE 6 : Miscellaneous compounds.

Plant	Family	Name	Structure	Melting point °C	Rotation	U.V. max. mμ	Ref.
1	2	3	4	5	6	7	8
Achlys bisexualis	-	Antheridiol	3β, 22ζ-OH, $\Delta^{5,24(28)}$, 7-OXO 23ζ-29 lactone	253-55	-	220	203
Achlys bisexualis	-	23-Deoxyantheridiol	3β-OH, $\Delta^{5,24(28)}$, 7-OXO, 22ζ-29-lactone.	265-70	-	230	204
Alangium lamarckii Th. W.	Alangiaceae	Compound -I	3β-OH, $\Delta^{5,22,25}$ (24S)	151-52	-44.0	210	205

Contd.....

TABLE 6 : (Contd....)

1	2	3	4	5	6	7	8
Aplopappus heterophyllus	Compositae	Compound -I	5α-H, 3β-OH, Δ8(14), 22	165-66	-	-	206
Celsia sommandetiana Vahl	Scrophulariaceae	Celsianol	3β-OH, $\Delta^{5,9(11)}$ (24ξ)	166-68	+3.1	-	207
Clerodendrum campbellii	Verbenaceae	Compound III	3β-O-, $\Delta^{5,22,25}$ (24S)	146	-37.8	-	208
Clerodendrum campbellii	Verbenaceae	-	3β-OH, 4α-CH_3, $\Delta^{7,25}$(24ξ)	142 (as acetate)	-	-	208
Coccinia indica	Cucurbitaceae	Compound I	Δ^{7}, 3-OXO	154-8	30	-	209
Coffea arabica	Rubiaceae	Coffeasterone	5α-H, 3β-OH, Δ8, 22(28) 4- = CH-CH_3	164	4.0	-	210
Zea mays	Gramineae	Compound II	5ξH, 3β-O-feruloyl	156-7	-	-	211
Corylus avellana Linn.	Betulaceae	Compound I	3α, 7α, 22α-OH, Δ^{5}	130-82	-	-	212

Contd.....

TABLE 6 : (Contd....)

1	2	3	4	5	6	7	8
Cryptocarya foveolata C.T. White	Lauraceae	Compound-II	3β-OH, Δ^{5},7-DXO	165-67	-	238	213
Echallium elaterium A. Rich.	Cucurbitaceae	Elasterol	5α-H, 3β-OH, $\Delta^{7,16,25(26)}$	167	8.2	-	214
Enteromorpha intestinalis	Chlorophyceae	28-Isofucosterol	3β-OH, $\Delta^{5,24(28)}$	133.5	-	-	215
Metasequoia glyptostroboides HU et Cheng.	Taxodiaceae	-	5α-H, 3,6-DXO	199	-	284	216
Metasequoia glyptostroboides HU et Cheng.	Taxodiaceae	-	5β-H, $\Delta^{4,22}$,3-OXO	-	-	-	216
Momordica charantia Linn.	Cucurbitaceae	Compound 3	5α-H, 3β-OH, $\Delta^{7,25}$	135-42	1.2	-	217
Momordica charantia Linn.	Cucurbitaceae	Compound 7	5α-H, 3β-OH, $\Delta^{7,22,25}$	157-61	11.0	-	217
Momordica charantia Linn.	Cucurbitaceae	Compound I	$\Delta^{5,25}$(24ζ)	133	-48.0	-	217
Muira puama	Aizoaceae	Compound I	5α-H, 3α-OH, $\Delta^{3,15}$, 20-CH_3	189-91	13.0	-	218

Contd.....

TABLE 6 : (Contd...)

1	2	3	4	5	6	7	8
Populus tremuloides	Salicaceae	Tremulone	$\Delta^{3,5}$,7-OXO, (24ξ)	111	-28.8	278	219
Rust fungi	-	-	5α-H, 3β-OH, $\Delta^{7,24(23)}$	142	-	-	220
Oryza sativa L.	Gramineae	-	3β-O-feruloyl, Δ^{5}	131-2	10.9	-	221
Samadera indica	Simarubaceae	Compound II	3-OXO, (24R)	157-9	41.4	-	222
Semanea saman. Merrill.	Leguminosae	α-Spinasterone	5αH, $\Delta^{7,22}$, 3-OXO	164-65	-	-	223
Sargassum ringoldianum	Phaeophyceae	Saringosterol	3β, 24ξ-OH, $\Delta^{5,28}$	160-61	-31.0	-	224
Solanum xanthocarpum Schrd & Wendl.	Solanaceae	Carpesterol	5αH, 3β-benzoyloxy, 22R-OH, Δ^{7},6-OXO, 4α-CH_3, (24R)	251	+67	233	159
Triticum vulgare Vill.	Gramineae	Substance II	$\Delta^{4,6,8(14),22}$, 3-OXO, (24ξ)	107-9	-	-	228

Contd.....

TABLE 6 : (Contd.....)

1	2	3	4	5	6	7	8
Vernonia anthelmintica willd.	Compositae	Compound I	5α-H, 3β-OH, Δ7, 24(28)	-	-	-	225
Vernonia anthelmintica willd.	Compositae	Vernosterol	5α-H, 3β-OH, Δ8(14), 15, 24(28)	-	-	-	226
Vernonia anthelmintica willd.	Compositae	Compound I	5ξ-H, 3β-OAc, Δ3, 14, 24(28)	-	-	-	227

TABLE 7 : Saponins

Plant	Family	Name	Melting point °C	Rotation	U.V. max. mμ	Ref.
Balanites roxburghii Planch.	Simarubaceae	5α-Stigmasta-7,22-dien-3β-glucoside	182-6	-	-	229
Blighia sapida KON.	Sapindaceae	Stigmasterol-fructoside	296-8	-	-	230
Maytenus senegalensis Moline.	Celastraceae	β-Sitosteryl-xyloside	-	-	-	231
Momordica charantia Linn.	Cucurbitaceae	Stigmasta-$\Delta^{5,25}$-dien-3β-glucoside*	285-90	-43.8	-	232
Wallenia yunquensis	Myrsinaceae	Stigmasterol-3α-L-arabinopyranoside	-	-	-	233

*Probably same as Stigmasta-$\Delta^{5,25}$-dien-3β-glucoside, m.p. 285-90° from Clerodendron infortunatim Linn.[234]

TABLE 8 : Unidentified compounds.

Plant	Family	Name	Groups	Melting point °C	Rotation	U.V. max. mμ	Ref.
Calendula officinalis Linn.	Compositae	Substance I	C_{10}-side chain, 24-ethylidene, Δ^7, 3β-OH	165	+ 6	-	235
Mimusops elengi Linn.	Sapotaceae	Fattyacidesters of α-spinasterol	Acid (oleic and Stearic)	-	+20	-	236
Monarda citriodora Gerv.	Labiatae	-	-	134-36	-238	-	237
Dryopteris filix and D. marginalis	Polypodiaceae	-	3β-OH, $\Delta^{5,11(7)}$ Stigmastane	137	-33.7	-	238
Schinus molle L.pirul.	Anacardiaceae	-	-	190-92	+29.6	212	239

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DISCUSSION OF CHAPTER I

(Chemical Investigation of *Physalis franchetii* Mast.)

Physalis franchetii Mast.(Physalis alkekengi var. franchetii. Hort.): N.O. Solanaceae: Local name Kaknaj

Physalis franchetii Mast.is a chinese lantern plant and differs from P. alkekengi chiefly in its greater size, making a plant 2 ft. tall, glabrous, petioles shorter and bearing Calices 2" diameter. It is a very showy plant and is very likely a variant of P. alkekengi[1].

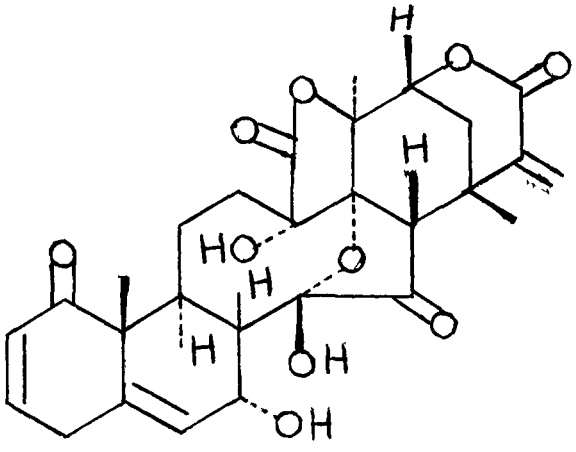
The fruits of P. franchetii were purchased from Lucknow's local market and got identified from National Botanic Gardens, Lucknow, India. The bazar fruits are spherical, yellowish brown in colour, 3 mm. to 12 mm. in diameter, much shrunken with a greyish mark left by the pedicel, closely packed with seeds that are flat, orbicular, but slightly irregular in outline about 3.5 mm. long and 3 mm. wide, pale coloured.

Kaknaj is a drug used chiefly in the Unani system of medicine as aparient, diuretic, and stimulant and is useful in diseases of the bladder[2]. It is also used as anthelmintic[3]. It is also reported to be efficacious in dropsy, rheumatism and intermittent fevers.

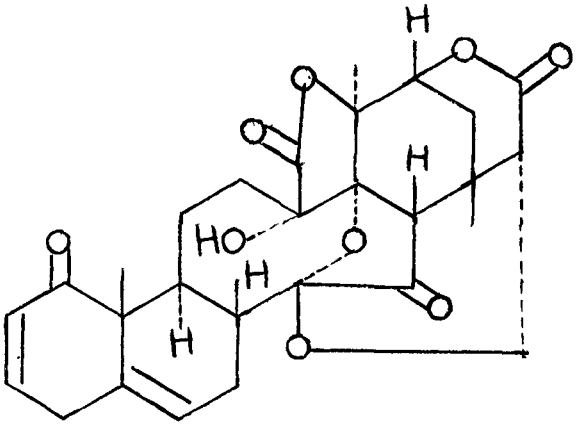
Nishimoto[4] reported the isolation of an alkaloid, 3 α(tigloyloxy)-tropane from Physalis alkekengi. Matsuura

et al.[5,6,7] have reported the isolation of three novel crystalline compounds physalin A, B and C with interesting 13,14, seco-16,24-cyclo-C-28 steroidal structures (I, (II) and (III) respectively from P. alkekengi var. franchetii. Further, Goynand et al.[8] have reported a flavonoid-glycoside, luteolin-7-β-D-glucoside from the leaves of P. alkekengi.

The isolation of various substances from other species of Physalis has also been reported. Harris[9] isolated an alkaloid from Physalis pallida Nuttal. Subramanian et al.[10] made a detailed chemical study on the chemical components of the herb of Physalis angulata and reported two crystalline compounds, of these one was identified as physalin B. Subsequently, they isolated three new bitter substances besides the known compounds, Physalin B from Physalis minima and Physalin A from Physalis peruviana.



(I)



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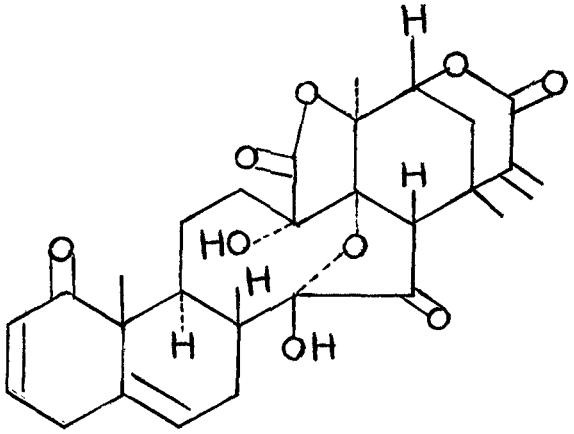


(III)

In view of the medicinal efficacy of Physalis franchetii Mast. and the isolation of chemically interesting compounds mentioned above, it was considered to be worthwhile to undertake a chemical investigation on the seeds of this plant.

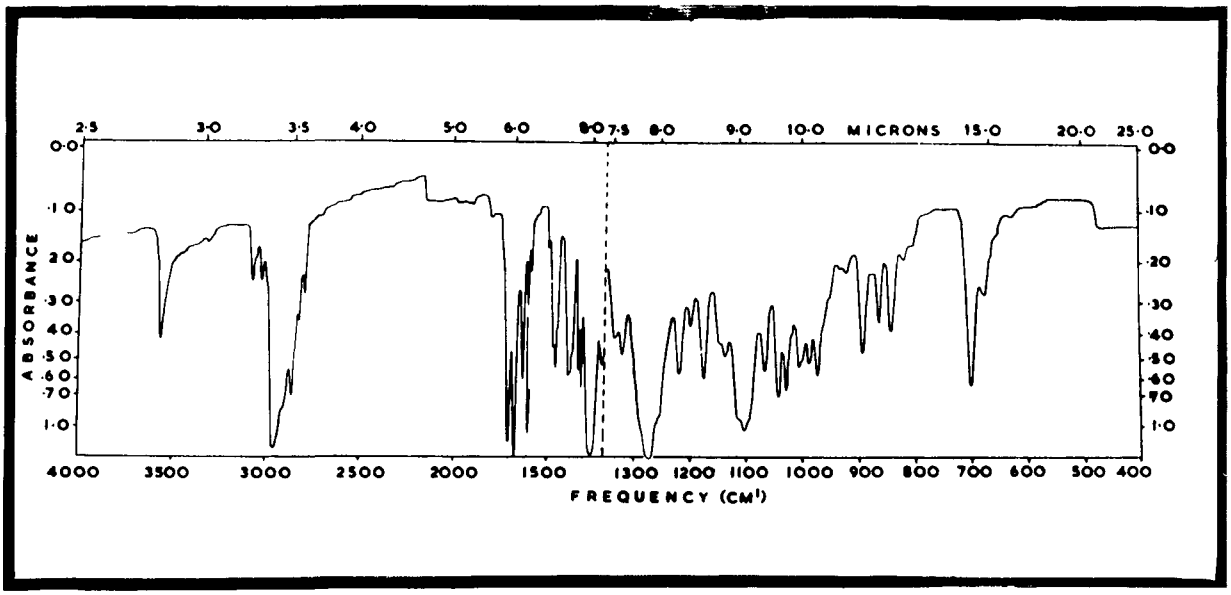
Isolation of Chemical Constituents

The powdered seeds of unripe fruits of *Physalis franchetii* (ast.were exhaustively extracted with hexane. On cooling, the hexane extract deposited a white crystalline mass, which was filtered. Its TLC on silica gel showed that it was a mixture of three compounds A, B and C in decreasing order of Rf values. The crude crystalline mixture was chromatographed over a column of neutral alumina. The hexane : benzene (1:1) eluate afforded Compound A in the pure form while the benzene eluate gave Compound B. Compound C was obtained from the benzene : Chloroform (3:1) eluate.

Characterization of Constituents

Structure of Compound A (Physanol A) :

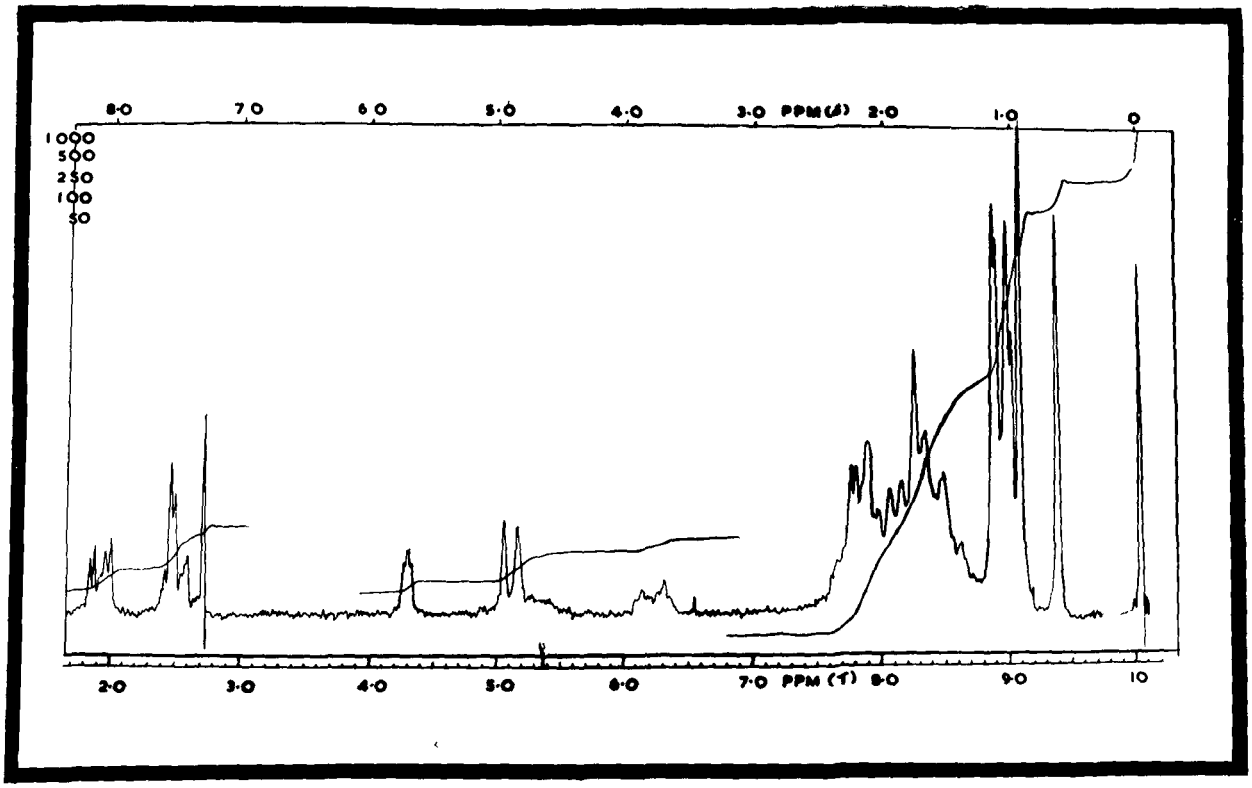
Physanol A crystallised as white colourless needles from chloroform : methanol, m.p. 234-36°, $[\alpha]_D$ = 60°, (C 1 %, chloroform), $C_{36}H_{50}O_4$, (M^+ 546). The positive Liebermann-Burchard test indicated that it was either a triterpenoid or a steroidal compound. Its steroidal nature was also evident from the IR and PMR spectra. The IR spectrum of physanol A manifested the presence of OH group (3560 cm^{-1}), aromatic nucleus (3073, 3030, 1604,



I R SPECTRUM OF PHYSANOL-A

1585, 1425 cm^{-1}), CH_3-, $-CH_2-$ (2958, 2865, 1462, 1388 cm^{-1}), aromatic ester grouping (1720, 1276, 1104 cm^{-1}), enone (1680, 1627 cm^{-1}) and a $>C = CH_2$ group (893 cm^{-1}). The presence of an aromatic ester group was also corroborated by its UV absorption at 237 nm. (ϵ 21,380) for a benzoyloxy group. An enone absorption in the UV region could not be observed because of the masking effect of the benzoyloxy absorption[11]. However, the high molar extinction coefficient of this band suggested the contribution of some other chromophore (enone) since there was only one benzoyloxy group in the molecule (vide infra). The PMR spectrum of physanol A exhibited signals for two quaternary methyl (3H each, s, at 0.65 and 0.93 ppm), two secondary methyl (3H each, d, J=7 Hz at 1.07 and 1.09 ppm), $-\underline{C}H-O-$(1H, dm, J = 10 Hz at 3.73 ppm), $-\underline{C}H-O-CO-$ (1H, broad m, at 4.66 ppm), $>C = C\underline{H}_2$ (two 1H, broad s, at 4.86 and 4.95 ppm), $-C = C\underline{H}-CO-$(1H, q, J = 1.5 Hz at 5.73 ppm) $C_6\underline{H}_5$-(2H, dd, J = 7 & 2 Hz at 8.1 ppm and 3H, m at 7.53 ppm).

Physanol A readily formed a monoacetate, m.p. 204-5°, $C_{38}H_{52}O_5$ (M^+588) and a monobenzoate m.p. 210-11°, $C_{43}H_{54}O_5$ (M^+650). The IR spectrum of the acetate did not show any hydroxyl absorption and the PMR spectrum displayed one acetyl singlet at 2.0 ppm. This suggested the presence of only one hydroxyl group in physanol A. Further, the $-\underline{C}H-O-$signal which appeared at 3.73 ppm in the PMR spectrum



PMR SPECTRUM OF PHYSANOL-A

of physanol A shifted to 5.25 ppm in the PMR spectrum of acetylphysanol A. This paramagnetic shift of more than 1 ppm on acetylation indicated the secondary nature of the hydroxyl group in physanol A. This inference was further confirmed by the oxidation of physanol A with Jones' reagent which afforded a mono-oxo derivative, m.p. 139-36°, $C_{36}H_{48}O_4$ (M^+544).

Since the IR and PMR spectra of physanol A indicated the presence of a vinylidene group, it was treated with m-chloroperbenzoic acid to form a monoepoxide derivative m.p. 225-30°, $C_{36}H_{50}O_5$ (M^+562). Epoxy physanol A did not show 893 cm^{-1} band but two new bands at 931 and 784 cm^{-1} for -C-O-C- stretching of the epoxide in the IR spectrum and its PMR spectrum was also lacking the two vinylic singlets at 4.86 and 4.95 ppm. Instead, there was a narrow multiplet corresponding to two protons at 2.63 ppm assignable to the methylene protons on one of the carbons bearing the epoxide. These findings confirmed the presence of a vinylidene group in the molecule.

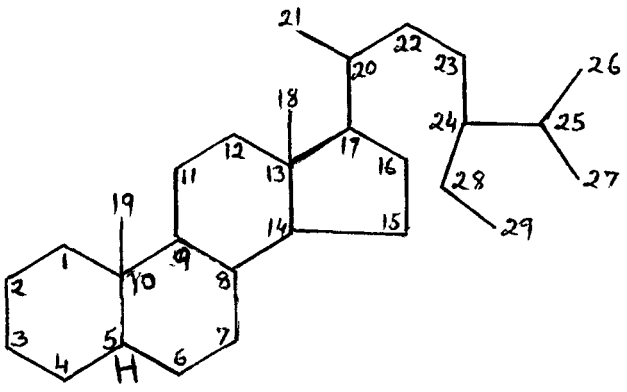
The hydrogenation of physanol A in ethylacetate in the presence of Adam's catalyst led to the formation of a dihydroproduct, m.p. 232-33°, $C_{36}H_{52}O_4$ (M^+548) in quantitative yield. However, when the hydrogenation was repeated in glacial acetic acid the dihydro as well as the tetrahydroderivatives were formed in a ratio of Ca 3:1.

The IR spectrum of dihydrophysanol A did not carry the vinylidene band at 893 cm^{-1} but still had the enone absorption. Its PMR spectrum also contained a signal only for a vinylic proton adjacent to a keto group (enone). Thus, in the formation of dihydrophysanol A, only the vinylidene bond had been reduced.

The tetrahydro derivative of physanol A, m.p. 128° $C_{36}H_{54}O_4$ (M^+550) did not show any vinylic proton signals in the PMR spectrum. It also did not exhibit vinylidene and enone absorptions in the IR spectrum which, however, showed a strong band at 1710 cm^{-1} for a six-membered ring ketone. This confirmed that in the tetrahydroproduct both vinylidene as well as enone double bonds had been reduced and that the enone group in physanol A was present in a six membered ring. The molecular formula of tetrahydro physanol A requires the presence of ten rings + double bonds, five of which are accounted for by the benzoyl group, one by the keto group and four by the steroid skeleton (vide infra). It may, therefore, be concluded that tetrahydro physanol A was completely saturated, hence physanol A contained only two double bonds.

The molecular formula of physanol A ($C_{36}H_{50}O_4$) and the products of its saponification ($C_{29}H_{46}O_3$, vide infra) in conjunction with its physicochemical data suggested

that physanol A was the benzoate of a C_{29} steroid (stigmastane type). The nature of the side chain as stigmastane type was also revealed by a 3H triplet (J = 7 Hz) at 1.1 ppm in the PMR spectrum (100 Mc) of physanol A which could only be assigned to the CH_3 protons of an ethyl group in the side chain. Further, in the mass spectra of physanol A and its debenzoylated derivatives there was a prominent loss of 139 mass units corresponding to the elimination of $C_{10}H_{19}$ side chain and in the mass spectra of dihydrophysanol A ($C_{36}H_{52}O_4$) as well as tetrahydrophysanol A ($C_{36}H_{54}O_4$) there was a corresponding loss of 141 mass units ($C_{10}H_{21}$) as in case of β-sitosterol which possesses a saturated C_{10} side chain. On the basis of above evidences it was concluded that the basic skeleton of physanol A was of stigmastane type(IV).



(IV)

since the physicochemical data of physanol A indicated the presence of a benzoyloxy group, it was saponified with mild alkali at ambient temperature for 48 hours and the products resolved into acidic and neutral fractions. The acidic fraction, on purification furnished fine colourless crystals, m.p. 122° and was identified as benzoic acid by preparation of its anilide and finally by its mixed melting point, superimposable IR spectrum and co-chromatography (TLC) with authentic benzoic acid. The neutral fraction showed two spots on TLC (Rf 0.29 and 0.36, 8 % methanol in benzene). Its chromatography over neutral alumina afforded two crystalline substances which were designated as debenzoylphysanol A and debenzoylphysanol A_1 in order of increasing Rf values.

<u>Debenzoylphysanol A</u> was obtained from methanol as colourless needles, m.p. 209°, $C_{29}H_{46}O_3$ (M^+ 442). In the UV region it showed an enone absorption at 245 nm (ϵ 12,250), which in physanol A was masked by the benzoyl absorption. The presence of an enone was corroborated by IR absorption bands at 1670 and 1633 cm^{-1}. Beside this, the IR spectrum also indicated the presence of OH (3533 cm^{-1}) and $>C=CH_2$ (890 cm^{-1}) functions in the molecule. The PMR spectrum of this product also exhibited a 1H quartet (J = 1.5 Hz) at 5.76 ppm and a pair of 1H broad singlets at 4.93 and 5.03 ppm assignable to $-\overset{|}{C}=C\underline{H}-CO-$ and $>C=C\underline{H}_2$ groups respectively. A 1H doublet of multiplets (J = 10 Hz) and a very broad

multiplet centred at 3.18 and 3.85 ppm were assignable to two methine protons adjacent to secondary hydroxyl groups. It is evident that debenzoylphysanol A contained all the functional groups present in physanol A except that the benzoate group was hydrolysed to a hydroxyl group, indicating that this is the normal product of saponification of physanol A.

Debenzoylphysanol A_1 crystallised from methanol-hexane as white colourless needles, m.p. 210-11°, $C_{29}H_{46}O_3$ (M^+ 442). It showed only an end absorption in UV at 203 nm (ϵ 8,142) but no enone absorption. Its IR spectrum also lacked the enone absorption bands but there was a band at 1703 cm^{-1} which could be assigned to a keto group in a six membered ring. The PMR spectrum of this product displayed the signals for two quarternary methyl groups, (3H each s, at 0.64 and 0.90 ppm), two methine protons on carbons bearing secondary hydroxyl groups (1H, d, J = 10 Hz at 3.86 ppm and 1H, broad m, at 3.25 ppm) and $\rangle C = CH_2$ (two 1H, broad s, at 4.9 and 4.903 ppm). The PMR spectrum, however, did not show any signal for $-\overset{|}{C} = CH-CO-$ proton but there was a 2 H multiplet centred at 3.0 ppm assignable to the two protons of a methylene flanked by a double bond and a carbonyl group ($-\overset{|}{C} = \overset{|}{C} - CH_2 - CO-$). Thus the double bond originally in conjugation with the carbonyl group in the parent compound (physanol A) has, in part, migrated out of conjugation to take up a tetrasubsti-

tuted position during alkaline hydrolysis to give debenzoyl-physanol A_1.

Position of Vinylidene Group :

In a steroid molecule the vinylidene group could not be accommodated in the skeletal part for want of an 'extra' carbon atom, it must, therefore, be located in the side chain. This inference also followed from the mass fragmentation of physanol A, which showed a facile cleavage of an unsaturated side chain ($C_{10}H_{19}$, 139 mass units)* from the $(M-C_6H_5COOH)^+$ fragment. The vinylidene double bond could thus be located Δ^{20}, Δ^{25}, or Δ^{23}, since the PMR spectrum of physanol A did not show characteristic signals for $-C(CH_3)=CH_2$ and $-CH=CH_2$ groups, positions Δ^{25} and Δ^{23} for the vinylidene group were ruled out. The vinylidene group was, therefore, assigned the Δ^{20} position.

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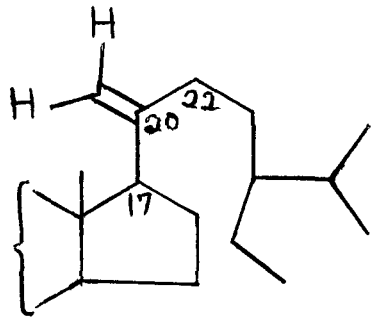
*In the mass fragmentation of steroid having a double bond at Δ^{22}, Δ^{24} and Δ^{25}, the side chain is eliminated alongwith two hydrogen atoms of the skeleton[12]. In case of physanol A, however, the elimination of side chain is undoubtedly not accompanied by loss of skeletal hydrogen atoms, since in the dihydro-product the loss of side chain fragment is two mass units higher.

Thus structure (V), accounts for the pair of singlets at 4.86 and 4.95 ppm, broadened by the allylic coupling with protons located at C_{22} and C_{17} in the PMR spectrum of physanol A.



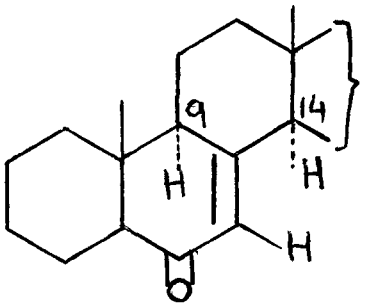
(V)

Position of Enone Group :

It has been pointed out earlier that physanol A possesses an enone chromophore of the type $-\dot{C} = CH-CO-$ in a six membered ring, it could, therefore, occupy any of the following positions in the steroid skeleton; Δ^4, 3-OXO; Δ^5, 7-OXO; Δ^7, 6-OXO; $\Delta^{9(11)}$, 12-OXO. The double bond of the enone system in physanol A should however, be placed so as to be able to take up a tetra-substituted position, as observed during the alkaline hydrolysis leading to the formation of debenzoylphysanol A_1. This requirement can be met only if the enone group is present either at $\Delta^{9(11)}$, 12-OXO or Δ^7, 6-OXO positions. The choice between these two positions was made on the

following grounds. The PMR signal of the vinylic proton adjacent to the carbonyl group in physanol A appeared as a quartet (J = 1.5 Hz) due to its allylic coupling with protons on γ-carbon atoms[13]. The splitting pattern (quartet) of the vinylic proton clearly suggested the Δ^{7}, 6-OXO position for the enone system, where it had two allylic protons available at C_9 and C_{14} to couple with. In the case of $\Delta^{9(11)}$, 12-OXO group the vinylic signal should have appeared as a narrow doublet (J = 1.5 Hz) due to its coupling with only one allylic proton available at C_9. Further confirmation of this position of the enone in physanol A was achieved by its sodium borohydride reduction which yielded a dihydroxy product m.p. 221-22°, $C_{36}H_{52}O_4$ (M^+ 548), transparent in the UV region. Its IR spectrum showed the presence of a trisubstituted double bond (793 and 815 cm^{-1}) but no carbonyl absorption. The PMR spectrum, however, showed, besides other signals, a vinylic proton signal (1H) as a broad doublet (J = 5.0 Hz) centred at 5.56 ppm and a 1H triplet (J = 5.0 Hz) centred at 4.26 ppm due to the methine proton adjacent to the newly generated hydroxyl group. The multiplicity of this methine proton originated from its coupling with the adjacent olefinic proton on one side and another proton on the other side (C_5-H in this case) and therefore confirmed Δ^{7}, 6-OXO as the position of enone (VI). The sodium borohydride reduction of $\Delta^{9(11)}$, 12-OXO- system

would have led to a 12-hydroxy $\Delta^{9(11)}$ group and the -CH-OH signal would have appeared as a doublet in the PMR spectrum, due to its coupling with only the adjacent olefinic proton.



(VI)

Position of the Hydroxyl Group :

The oxidation product of physanol A displayed an IR absorption band at 1703 cm^{-1} characteristic of a six membered ring ketone. Thus the hydroxyl group which gave rise to the keto group on oxidation, was located in six-membered ring. Further, the PMR spectrum of keto-physanol A showed a 2H singlet at 3.11 ppm and another sharp singlet at 2.1 ppm, probably representing 1H, sticking out of a crowd of other signals. Obviously, these downfield signals were due to the protons on carbon atoms adjacent to the carbonyl group. The singlet nature of both these signals required that these γ-protons should have

no vicinal protons to couple with. The only position for the keto group that satisfied this requisite is C_{11}. The assignment of singlets at 2.1 and 3.11 ppm would then be consistent with the C_9 methine and C_{12} methylene*. Thus, the hydroxyl group was located at C_{11} in physanol A. As regards its configuration, the 11β configuration would render this hydroxyl group too highly hindered (due to its 1,3 interaction with 18 and 19-methyl groups) to be easily acetylated. The acetylation of physanol A, however, proceeded smoothly with acetic anhydride in pyridine at room temperature. This fact suggested that the hydroxyl group had a α-configuration. Moreover, the methine proton adjacent to the hydroxyl group appeared as a doublet (J = 10 Hz) of multiplets. The magnitude of J value indicated that this proton should be β-axial hence the adjacent hydroxyl group should be α-equatorial.

---

*In the PMR spectrum of 5α-androstan-11- one the C_{12} methylene signal appears as a singlet at 2.27 ppm but the C_9 methine signal lies burried in the methylene envelope. Further, a pair of broad resonances at 2.45 ppm have been assigned to 1β proton, which falls in the deshielding zone of the 11-keto group[14]. In case of 11-keto physanol A, however, the 12-methylene and 9-methine protons resonate at much lower fields due to the Δ^7, 6-OXO grouping which deshields these protons. However, the signal due to 1β proton was not clearly distinguishable because of some other resonances in that region.

Position of Benzoyloxy Group :

Since the debenzoylated products of physanol A did not form an acetonide and were also inert to the action of sodium periodate, the possibility of a 1, 2 diol group in these products was ruled out. Thus the benzoyloxy and hydroxyl groups in physanol A were not present on adjacent carbon atoms. It may be recalled that the PMR spectrum of physanol A had a smeared type broad multiplet at 4.66 ppm due to a proton adjacent to the benzoyloxy group but in the spectrum of the debenzoylated physanol A, this signal shifted downfield to 3.13 ppm. The position and shape (broad, smeared type) of this methine multiplet in the PMR of debenzoylphysanol A and A_1 was very characteristic of 2β-axial or 3α-axial proton of 2α or 3β hydroxy steroids respectively[15]. Thus the benzoyl group in physanol A could be situated either at 2α or 3β position. In view of the ubiquitous presence of an oxygen function at C_3 in natural steroids, the benzoyloxy group was considered to be present at 3β position.

The placement of functional groups in physanol A was further confirmed by calculating the shift values of 18-methyl and 19-methyl groups of physanol A and its various derivatives taking 5α-stigmastane as the basic skeleton and comparing these values with the corresponding observed values. The calculated values were found to be in excellent

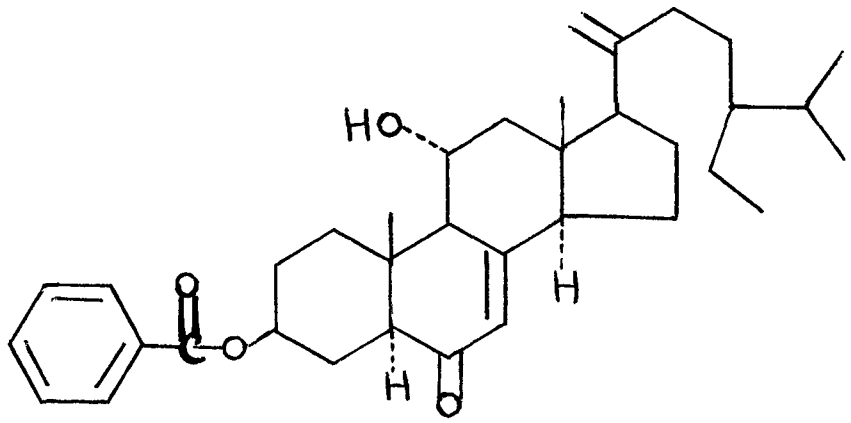
agreement with the observed values and are listed in table 1.

On the basis of above evidences structure (VII) was proposed for physanol A.

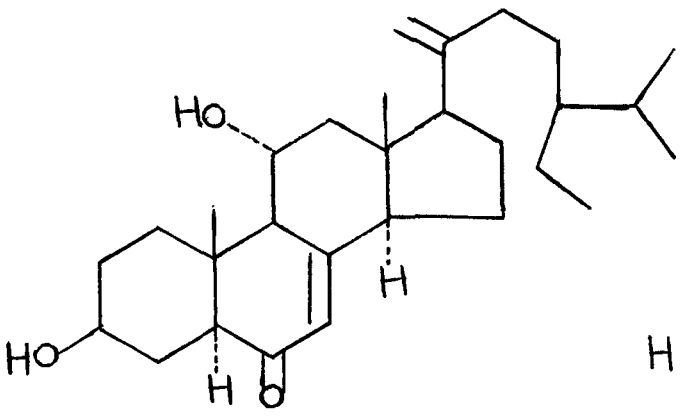
Since the debenzoylphysanol A was the normal product of saponification of physanol A, its structure would be (VIII).

In debenzoylphysanol A_1 the double bond of the enone (Δ^7, 6-OXO) had migrated to a tetrasubstituted position, $\Delta^{8(9)}$ or $\Delta^{9(14)}$. In order to settle this point debenzoylphysanol A was oxidised to the 3,6,11-triketoproduct, m.p. 191°. $C_{29}H_{42}O_3$ (M^+438). In the case of a $\Delta^{8(9)}$ bond, oxidation was anticipated to give an enone system, $\Delta^{9(9)}$, 11-OXO. The triketo product did not, however, show any enone UV absorption indicating that the tetrasubstituted double bond was situated at the $\Delta^{9(14)}$ position. Moreover, the $\Delta^{9(9)}$ bond in debenzoylphysanol A_1 would have rendered the splitting pattern of the PMR signal of the 11β-proton different from that of physanol A. This, however, remained unchanged, thus confirming the position of the double bond. The shifting of the double bond to $\Delta^{9(14)}$ position out of carbonyl conjugation in physanol A during alkaline hydrolysis is not surprising since it would stabilise ring D by relieving some of the strain inherent in a five membered ring[16]. The migration of

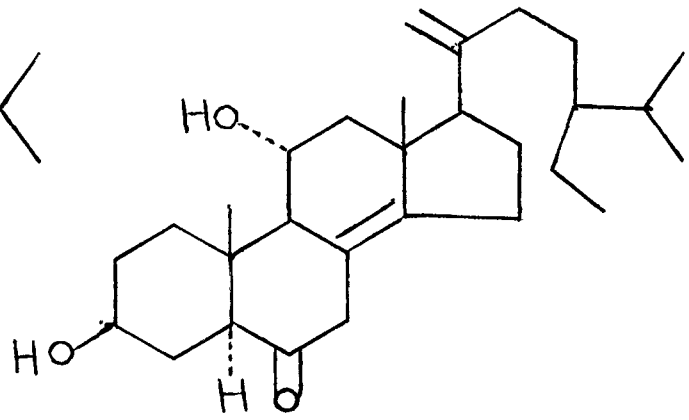
a Δ^7 bond to $\Delta^{8(14)}$ is also observed in case of several other sterols viz. carpesterol[17]. Thus the structure of debenzoylphysanol A_1 is (IX).



(VII)

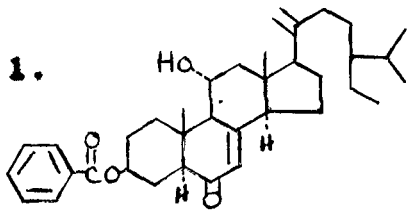
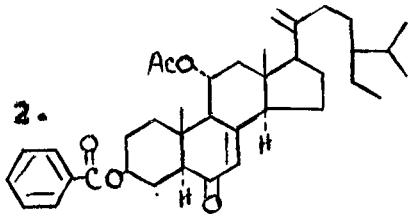
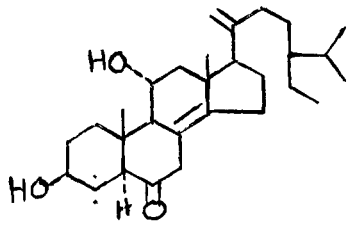
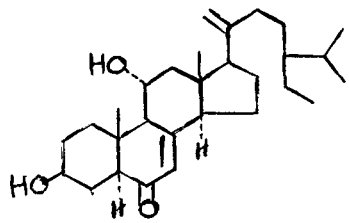


(VIII)



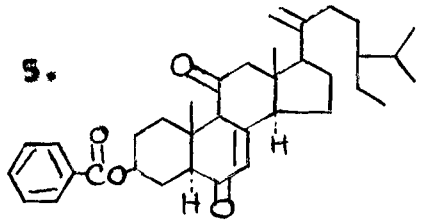
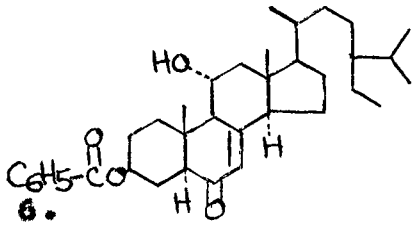
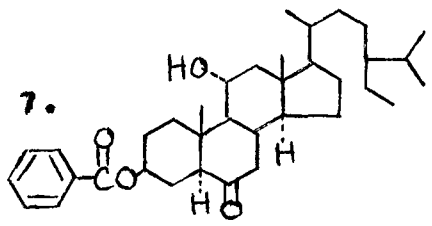
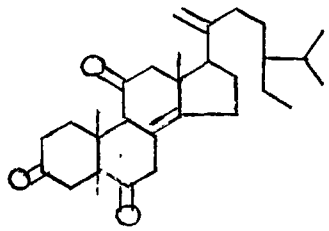
(IX)

TABLE 1 : Chemical Shifts of 19-methyl and 18-methyl protons of physanol A and its derivatives.

Sl No	Structure	19-Methyl			18-Methyl		
		Observed CPS	Calculated CPS*	Diff	Observed CPS	Calculated CPS	Diff.
1.		55.5	54.5	1.0	33.5	37.0	1.5
2.		55.5	53.0	2.5	33.0	39.0	-1.0
3.		54	46.5	7.5	38.5	54.5	-16.0**
4.		52.0	52.0	0.0	38.0	37.0	1.0

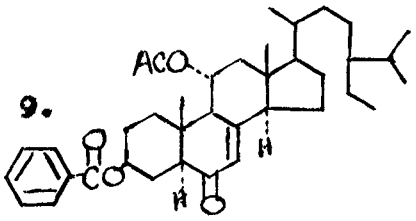
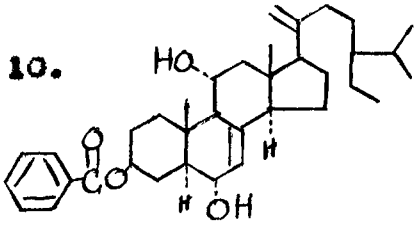
Contd.....

TABLE 1 : (Contd...)

Sl No	Structure	19-Methyl			18-Methyl		
		Observed cps	Calculated cps*	Diff.	Observed cps	Calculated cps	Diff.
5.		62.0	59.5	2.5	34.0	33.0	1.0
6.		55.0	52.5	2.5	37.0	35.5	1.5
7.		54.0	52.5	1.5	44.0	42.5	1.5
8.		67.0	64.5	2.5	51.5	52.0	-0.5

Contd....

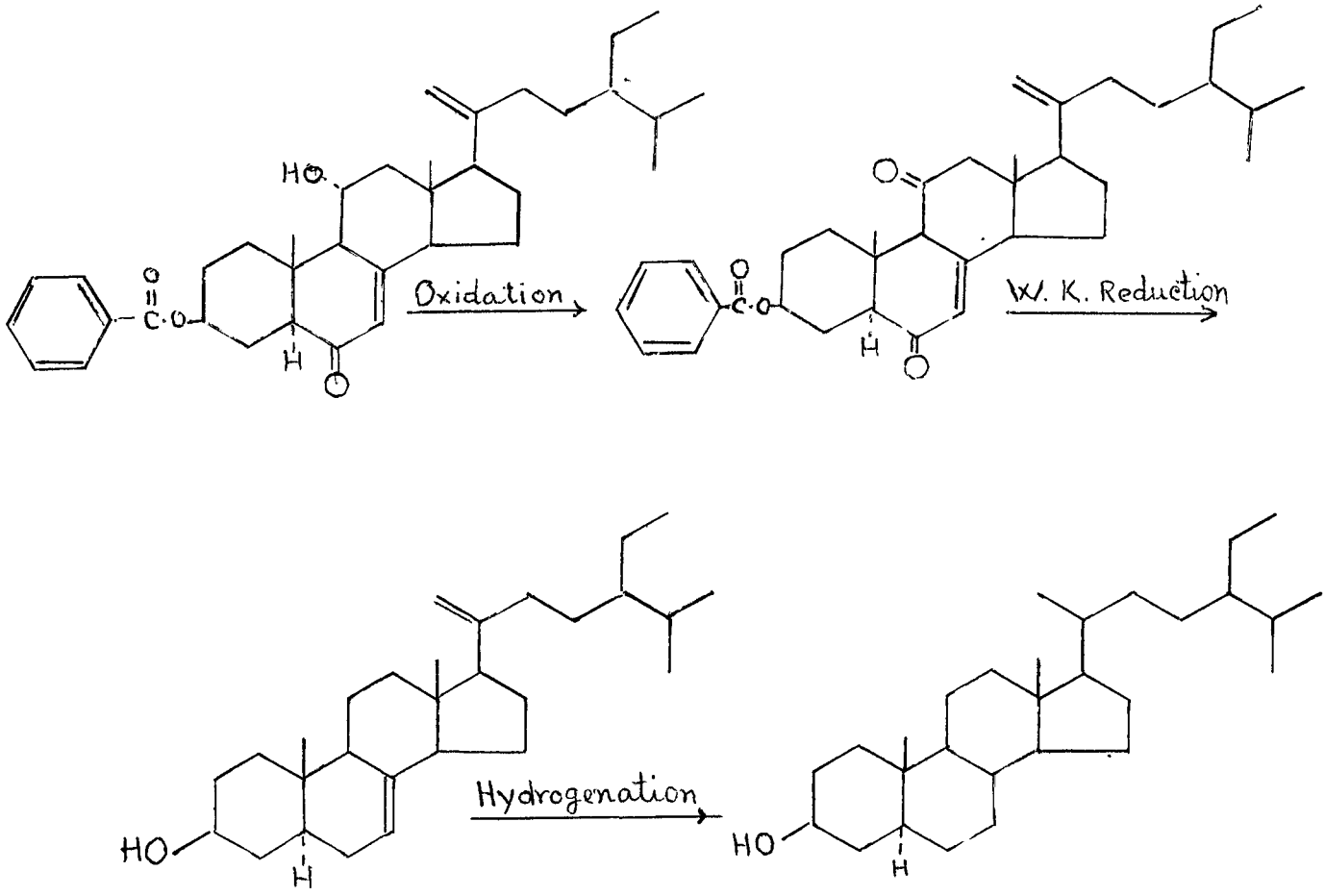
TABLE 1 : (Contd...)

Sl No	Structure	19-Methyl			18-Methyl		
		Obser-ved cps	Calcu-lated cps*	Diffi	Obser-ved cps	Calcu-lated cps	Diffi.
9.		54.5	52.5	2.5	37.5	37.5	0.0
10.		68.5	69.5	-1.0	39.0	38.5	0.5

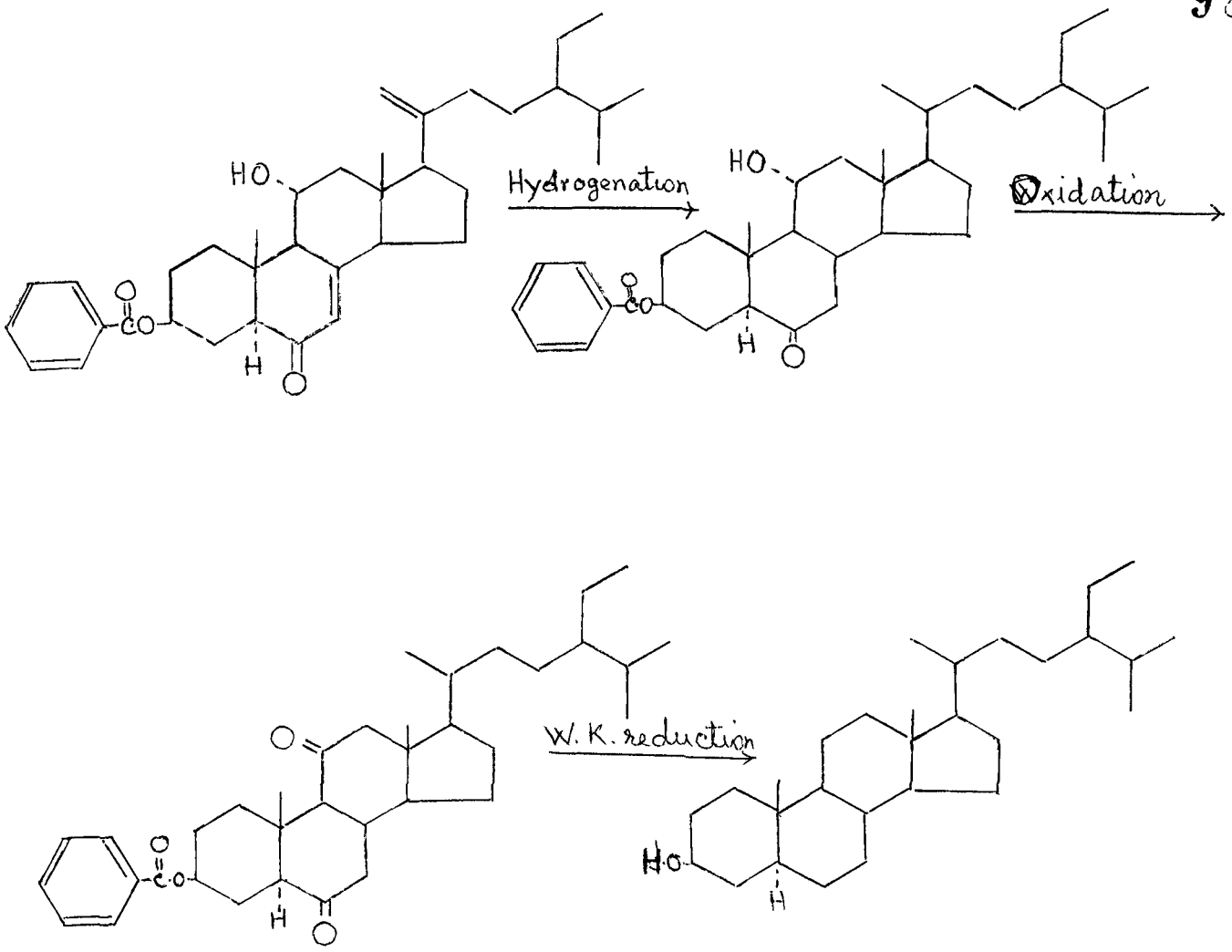
*The additive shift contribution of 3β-benzoyloxy group was obtained from the difference in chemical shifts of 19-methyl and 18-methyl protons of β-sitosterol, stigmasterol, α-spinasterol and those of the corresponding benzoates. The shift contribution of $\Delta^{2,3}$ bond was obtained by subtracting the 19-methyl and 18-methyl chemical shifts of dihydrophysanol A from those of physanol A. Rest of the shift values were taken from the literature[18].

**Anomaly is probably due to change in the geometry of the molecule on introduction of $\Delta^{3(14)}$ bond[19].

Although the structure of physanol A is based on the well founded chemical and physicochemical evidences, it has not yet been correlated with any known stigmastane derivative in order to provide a rigorous proof of its skeleton. During the course of investigations reported, attempts were, however, made to correlate its structure with stigmast-3-ol, or stigmastane through the following reaction schemes (1 - 3).

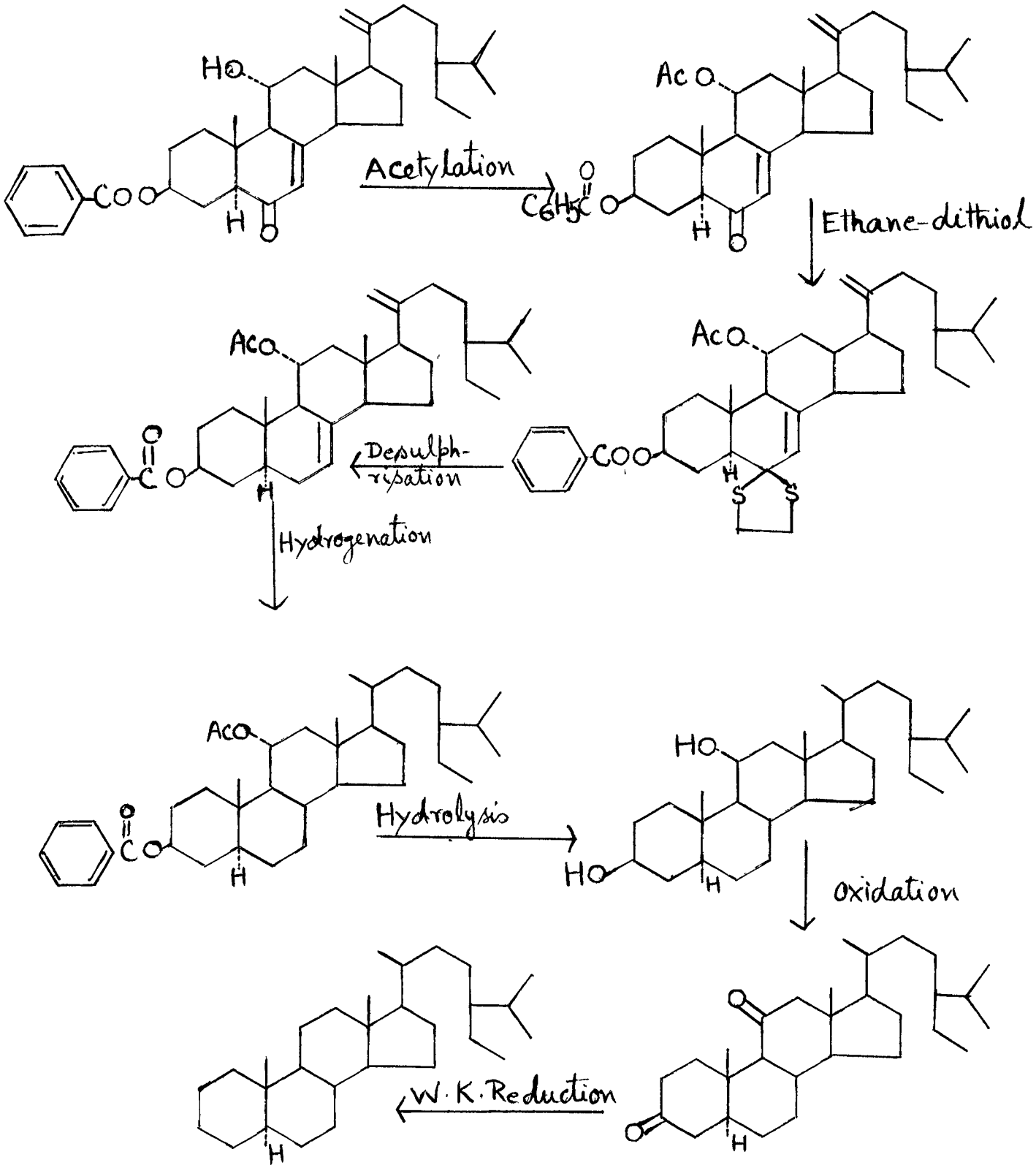


Scheme 1



Scheme 2

In pursuance of scheme -1 the Wolff-Kishner reduction of 11-oxophysanol A was carried out using Barton's modification[20] as well as Nagata's modification[21], but the reaction product was found to be a complicated mixture from which no satisfactory product could be isolated. In view of the above failure it was considered worthwhile to hydrogenate the conjugated double bond of physanol A, prior to its oxidation followed by Wolff-Kishner reduction (scheme-2).


HO
Acetylation
AcO
Ethane-dithiol
AcO
Depsulph-
risation
AcO
Hydrogenation
AcO
Hydrolysis
HO
HO
oxidation
W·K·Reduction

Scheme 3

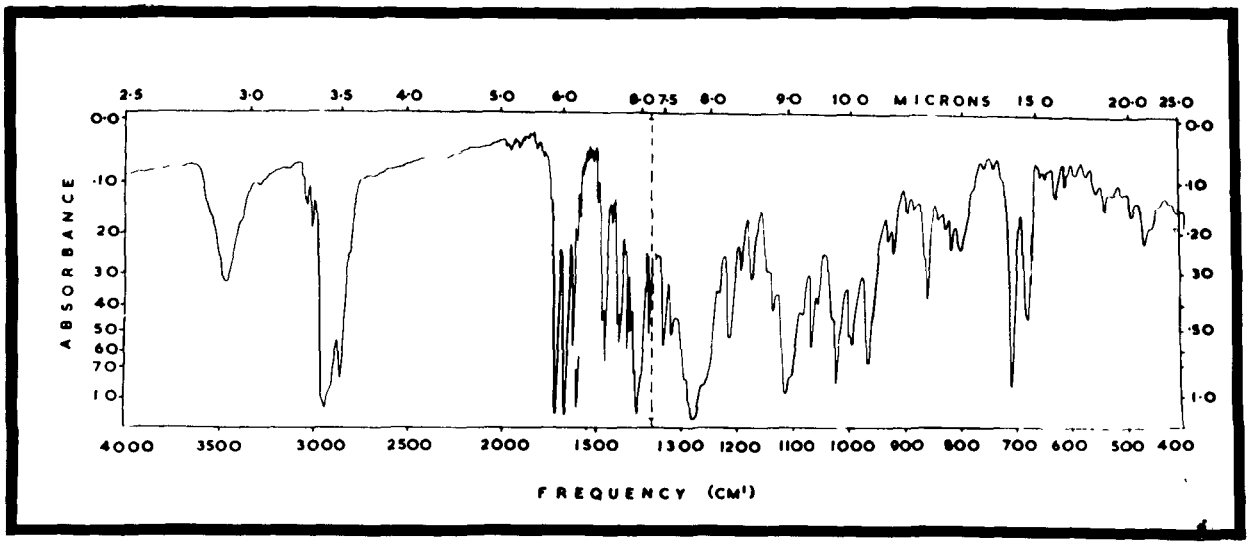
The hydrogenation of physanol A in presence of palladium on carbon in ethyl acetate and Adam's catalyst in ethyl acetate yielded only dihydrophysanol A. However, in presence of Adam's catalyst in acetic acid its hydrogenation resulted in the formation of only a minute amount of the tetrahydroproduct alongwith dihydrophysanol A. The latter catalytic reduction was therefore, repeated in the presence of traces of perchloric acid, but the product was a mixture of dihydrophysanol A and a number of other unidentified products. Scheme-2 also, therefore, had to be given up. In scheme-3 the removal of the conjugated keto group was sought through the preparation of a thioketal followed by desulphurisation. But on treatment of 3-acetyl-physanol A with ethanedithiol under conditions recommended for the preparation of thioketal of a conjugated keto group[22] (in presence of BF_3-etherate) and with ethanedithiol and p-toluenesulphonic acid in benzene with azeotropic removal of water, a gummy mixture of a large number of products (TLC) was obtained. Paucity of the starting material did not permit further experimentation.

Interestingly, physanol A constitutes the first example of steroids with a Δ^{20} bond ever isolated from a natural source. Even in the field of tetracyclic triterpenoids, eglalol is the only natural product possessing a Δ^{20} bond known so far. This is also the first example of natural stigmastane type of compound having an 11-oxygen function.

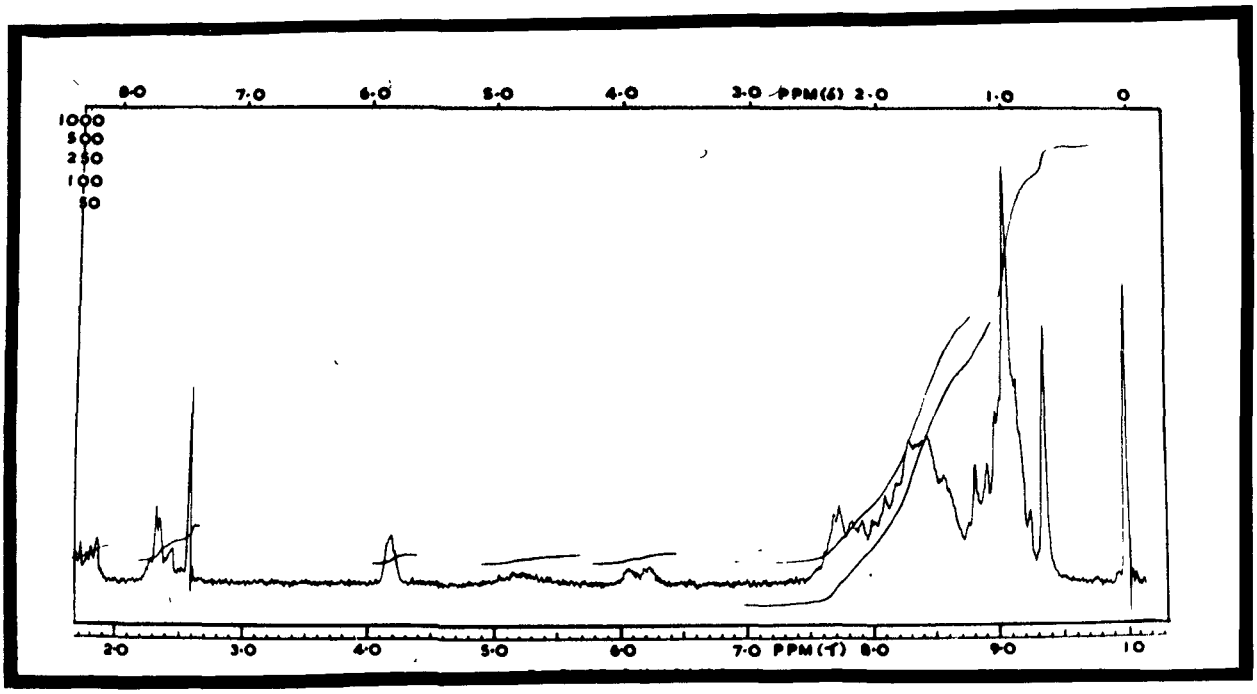
Structure of Compound B (Physanol B) :

Physanol B crystallized as white colourless needles from chloroform-methanol, m.p. 232-33°, $[\alpha]_D$ = +63° (c, 1% chloroform), $C_{36}H_{52}O_4$ (M^+ 548). It also exhibited a positive Liebermann-Burchard test indicating that it was either a triterpenoid or a steroid. However, its IR and PMR spectra indicated a steroidal nature. The IR spectrum manifested the presence of OH group (3430 cm^{-1}), aromatic nucleus (3035, 3010, 1604, 1580 and 1460 cm^{-1}), CH_3-, $-CH_2-$ (2935, 2860, 1380 cm^{-1}), aromatic ester grouping (1720, 1275, 1112 cm^{-1}), enone (1680, 1630 cm^{-1}) no absorption for a $C = CH_2$ group (893 cm^{-1}). The PMR spectrum of physanol B exhibited signals for two quaternary methyl (3H each, s, at 0.62 and 0.91 ppm), two secondary methyl (3H each, d, J = 7 Hz, at 1.07 and 1.03 ppm), $\underline{CH}$-O-(1H, dm, J = 10 Hz at 3.65 ppm), $-\underline{CH}$-O-CO-(1H, broad m, at 4.73 ppm), $-C = \underline{CH}$-CO-(1H, q, J = 1.5 Hz at 5.31 ppm). $C_6\underline{H}_5$-(2H, dd, J = 7 and 2 Hz at 8.1 and 3H, m, at 7.53 ppm).

Physanol B formed a monoacetate, m.p. 232°, $C_{38}H_{54}O_5$ (M^+ 590) and a monobenzoate, m.p. 230-31°, $C_{43}H_{56}O_5$ (M^+ 652), which showed no hydroxyl absorption in the IR spectrum. This suggested the presence of only one hydroxyl group in physanol B. The lowfield shift of more than 1 ppm on acetylation and benzoylation indicated the secondary nature of the hydroxyl group.

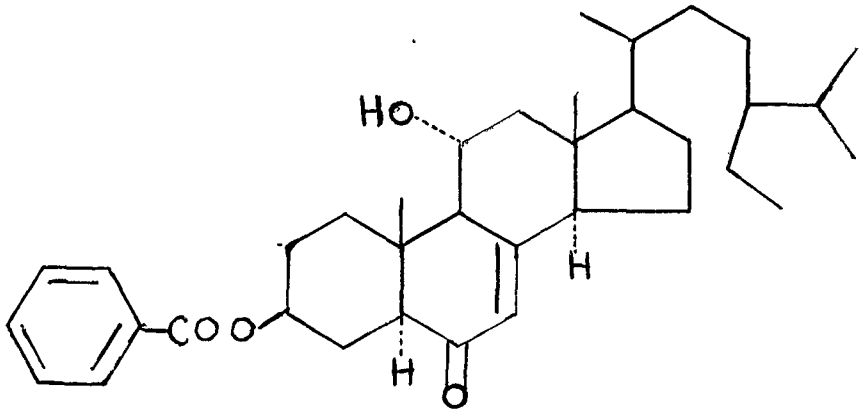


I R SPECTRUM OF PHYSANOL-B



PMR SPECTRUM OF PHYSANOL-B

The molecular formula of physanol B indicated a difference of one double bond from physanol A. Its mass spectrum also displayed a prominent peak m/e 235 formed by the loss of 141 mass units from $(M - C_6H_5COOH)^+$ peak representing the elimination of a saturated C_{10} side chain. Thus the side chain of physanol B did not carry any double bond. This fact was also substantiated by the lack of vinylidene absorption in the I.R spectrum and absence of characteristic vinylidene signals in the PMR spectrum. It was, therefore, concluded that physanol B was a dihydro derivative of physanol A. It was confirmed by superimposing the I.R spectrum of physanol B on that of dihydro-physanol A, their mixed melting points and co-chromatography (TLC). The structure of physanol B was, therefore, proposed as (X).



(X)

Compound C (β-sitosterol) :

Compound C was crystallised from methanol-chloroform, m.p. 136-37°. It gave positive Liebermann-Burchard test and formed an acetyl derivative m.p. 125-25°. From its mixed melting point, superimposable IR spectra and co-chromatography with an authentic sample, its identity was confirmed as β-sitosterol.

EXPERIMENTAL OF CHAPTER X

All the melting points reported are uncorrected. Optical rotations of 1 % solutions of the compounds were measured by Carl-Zeiss 368038 Germany. The UV spectra were taken on Perkin-Elmer 202 Ultraviolet - visible spectrophotometer in ethanol or methanol. The IR spectra recorded on a Perkin-Elmer 337 Grating Infrared Spectrophotometer in KBr. The PMR spectra were taken on a Varian A-60 D machine in $CDCl_3$ with TMS as internal standard. Mass spectra were run on Hitachi RMU 6E Mass Spectrometer.

The air-dried, powdered seeds (15 kg) of the fruits of Physalis franchetii Mast. were exhaustively extracted with petrol ether (60-80°), the total extract was concentrated at 50° under reduced pressure. On cooling for seven days the concentrate deposited a white crystalline mass, which was filtered and thoroughly washed with hexane. The crude crystallisate (8 g) on TLC over silicagel showed that it was a mixture of three major compounds A, B and C with Rf values 0.51, 0.35 and 0.12 respectively (Benzene + 2 % methanol). The crude crystalline mixture was chromatographed over neutral alumina (activity 3, 400 g),

two hundred and fifty fractions of 100 ml each, were collected. The results are recorded in table 1.

TABLE 1 : Chromatography of Crude Crystalline mass (3 g)

Fraction Number	Eluant	Weight (g)	TLC*
1- 10	Petrol ether	-	-
11- 21	Petrol ether; Benzene (3:1)	-	-
22- 60	Petrol ether : Benzene (1:1)	2.17	A
61-158	Petrol ether : Benzene (1:3)	3.25	A, B
158-188	Benzene	0.33	B
189-229	Benzene : Chloroform (3:1)	0.81	B, C
230-250	Benzene : Chloroform (1:1)	0.35	C

*Here and in the subsequent work following systems have been used to locate the spots on TLC over silica gel G; Benzene + 2 % methanol and Benzene + 3 % methanol. (A 1 % solution of ceric sulphate in 2 N H_2SO_4 has been used as a general spray reagent).

Fractions 22-60 containing compound A were combined and concentrated. The residue (2.17 g) was charcoaled and repeatedly crystallised from chloroform-methanol to give fine colourless needles of compound A, m.p. 234-36° (1.3 g).

The combined fractions 159-188 were evaporated to dryness and the residue (0.33 g) on crystallization from chloroform-methanol furnished colourless needles of compound B, m.p. 232-33°, 0.210 g.

The fractions 230-250 (0.35 g) on keeping with chloroform-methanol deposited colourless crystals of Compound C, m.p. 136-37°, 0.215 g.

Compound A (Physanol A)

Physanol A crystallized from chloroform-methanol as colourless needles, m.p. 234-36°, $[\alpha]_D$ = 60° (C 1 %, chloroform), Rf 0.51 (Benzene + 2 % methanol). It gave a positive Liebermann-Burchard test; $\lambda_{max.}^{EtOH}$ 237 nm (ε, 21,330); $\nu_{max.}^{(KBr)}$ 3560, 3075, 3030, 2953, 2865, 1720, 1630, 1627, 1604, 1585, 1462, 1425, 1383, 1319, 1276, 1216, 1196, 1174, 1104, 1066, 1043, 1023, 1005, 938, 973, 893, 853, 842, 700 cm^{-1}, PMR : ppm 0.65, 0.93 (3H each, s, 2 x quaternary $C\underline{H}_3$); 1.07, 1.09 (3H each, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 3.73 (1H, dm, J = 10, $-C\underline{H}-OH$); 4.66 (1H, m, $-C\underline{H}-OCOC_6H_5$); 4.86 and 4.95 (two 1H, broad s, $>C = C\underline{H}_2$); 5.73 (1H, q, J = 1.5 Hz, $-C = C\underline{H}-CO$); 8.1 and 7.53 (2H, dd, J = 7 & 2 Hz and 3H, m, respectively, $-C_6\underline{H}_5$). Found : C, 79.07; H, 9.32; $C_{36}H_{50}O_4$ requires : C, 79.1; H, 9.1 per cent. Mass : M^+546, 424 (M-122), 285 (M-122-139), 243 (285-42).

Mono-O-acetylphysanol A : Physanol A (75 mg) was allowed to stand overnight with dry pyridine (0.5 ml) and acetic-anhydride (0.5 ml). The reaction mixture was then worked up as usual. The acetylated product was purified by coloumn chromatography on alumina, crystallised from chloroform-methanol as colourless needles, m.p. 204-5°, $[\alpha]_D$ = 42° (C 1 %, chloroform), $\nu_{max.}^{(KBr)}$, 2950, 2862, 1725, 1703, 1680, 1623, 1594, 1573, 1450, 1378, 1322, 1310, 1272, 1196, 1179, 1140, 1110, 1069, 1023, 1000, 965, 935, 923, 895, 853, 820, 708 cm^{-1}, NMR : ppm. 0.621, 0.93 (2 x 3H, s, 2 x quaternary CH_3), 1.05 (2 x 3H, d, J = 7 Hz, 2 x secondary CH_3), 2.0 (3H, s, $-OCO.CH_3$), 4.76 (1H, broad m, $-\overset{1}{C}H-O.COC_6H_5$), 4.85, 4.91 (1H, each, s, $-\overset{1}{C} = CH_2$), 5.25 (1H, td, J = 6 and 2.5 Hz. $-\overset{1}{C}H-OAc$), 5.93 (1 H, q, J = 1.5 Hz, $-\overset{1}{C} = CH-CO$), 8.25, 7.68 (2H, dd, J = 7 and 2 Hz; 3 H, m, respectively, $-C_6H_5$); Found : C, 77.42; H, 9.01, $C_{39}H_{52}O_5$ requires : C, 77.55; H, 8.34 per cent, M^+538.

Mono-O-benzoylphysanol A : Physanol A (50 mg) was treated with benzoyl chloride (0.5 ml) in dry pyridine (0.5 ml) and the reaction mixture was allowed to stand overnight. It was worked up in usual manner and purified by chromatography on alumina. The product crystallised from chloroform-methanol as colourless needles, m.p. 210-11°, $[\alpha]_D$ = 22° (C 1 %, chloroform) $\lambda_{max.}^{EtOH}$ 231 nm,

(ε,37, 620); $\nu_{max.}^{(KBr)}$; 2907, 1706, 1656, 1623, 1451, 1377, 1368, 1314, 1274, 1213, 1173, 1112, 1071, 1027, 971, 893, 872, 809, 707, 685 cm^{-1}, PMR ; ppm ; 0.65; 0.93 (2 x 3H each, s, 2 x quaternary $C\underline{H}_3$); 4.73 (1H, m, $-\overset{|}{C}\underline{H}-O.CO.C_6H_5$); 4.88 (2H, s, $-\overset{|}{C} = C\underline{H}_2$); 5.51 (1H, m, $-\overset{|}{C}\underline{H}\ O-COC_6H_5$); 5.85 (1H, q, J = 1.5 Hz, $-\overset{|}{C} = C\underline{H}-CO-$); 7.51-7.8, 8.05-8.35 (6H and 4H respectively, 2 x $C_6\underline{H}_5$). Found ; C, 80.53; H, 8.65; $C_{43}H_{54}O_5$. Requires ; C, 80.62; H, 8.43 per cent, M^+650.

Oxidation of physanol A ; Physanol A (100 mg) was dissolved in acetone and 3N chromic acid (Jones' reagent) was added dropwise until an orange colour persisted. After allowing to stand for 10 minutes at room temperature, acetone was removed under reduced pressure and the residue was diluted with water and extracted with ether. The ether solution was washed thoroughly with dilute solution of sodium hydrogen carbonate and water, dried and evaporated. The oxidation product (80 mg) was purified by silica gel chromatography using benzene as the eluant. The purified product on crystallisation from chloroform-methanol yielded colourless crystalline needles (40 mg), m.p. 185-86°, $[\alpha]_D$ = 25° (C 1 %, chloroform), Rf 0.32 (Benzene + 0.8 % methanol), λ_{max}^{MeOH} 234 (ε,19,040); $\nu_{max.}^{(KBr)}$; 2950, 1708, 1669, 1630, 1598, 1450, 1380, 1330, 1314, 1275, 1220, 1200, 1180, 1115, 1095, 1063, 1028, 973, 895, 869, 700 cm^{-1} ;

PMR ; ppm. 0.56, 0.88 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$), 1.05 (2 x 3H, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 2.1 (~ 1H, s, $-C\underline{H}-CO-$); 3.11(2H, s, $-CO-C\underline{H}_2-$); 4.68(1H, m, $-C\underline{H}-O-CO-C_6H_5$); 4.76, 4.95 (1H each, s, $-\overset{|}{C} = C\underline{H}_2$); 5.65 (1H, q, J = 1.5 Hz, $-\overset{|}{C} = C\underline{H}-CO-$); 7.3-7.55, 8.5-8.11 (3H and 2H respectively, $-C_6\underline{H}_5$) Found : C, 79.35; H, 9.19; $C_{36}H_{43}O_4$ requires : C, 79.41; H, 8.32 per cent, M^+544.

Epoxidation of physanol A : Physanol A (160 mg) was dissolved in chloroform (15 ml) and to it was added m-chloroperbenzoic acid (130 mg). The reaction mixture was allowed to stand overnight. Excess of m-chloroperbenzoic acid was destroyed by dropwise addition of a solution of sodium metabisulphite (KI-Starch test) and then the reaction mixture was washed with water, dried and evaporated to dryness. The residue (160 mg) showed on major spot on TLC (Benzene + 1 % methanol) and was purified by preparative TLC. The lower zone was crystallised from chloroform-methanol as colourless needles, m.p. 225-30°, $\nu_{max.}^{(KBr)}$, 3584, 2967, 2882, 1725, 1683, 1647, 1621, 1604, 1468, 1391, 1368, 1340, 1323, 1275, 1225, 1206, 1185, 1157, 1110, 1071, 1046, 1032, 1011, 989, 976, 931, 875, 849, 784, 709, 686 cm^{-1}, PMR ; ppm. 0.63, 0.93 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$); 1.08 (2 x 3H, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 2.68 (2H, s, $-C\underline{H}_2$); 4.0 (1H, dm, J = 10Hz, $-C\underline{H}-OH$); 4.76 (1H, m, $-C\underline{H}-OCOC_6H_5$); 5.76 (1H, q,

J = 1.5 Hz, $-\overset{1}{C}$ = C$\underline{H}$-CO-), 7.3-7.71, 8.1-8.35 (3H and 2H respectively, $C_6\underline{H}_5$). Found : C, 76.79; H, 8.91 $C_{36}H_{50}O_5$ requires : C, 76.86; H, 8.89 per cent, M^+562.

Catalytic hydrogenation of physanol A : Physanol A (300 mg) was dissolved in glacial acetic acid (200 ml) and hydrogenated in the presence of platinum catalyst at atmospheric pressure for 8 hours. The reaction mixture was filtered and freed of acetic acid at 50° under reduced pressure. The residue (290 mg) showed two spots on TLC, Rf. 0.4 and 0.12 (Benzene + 0.8 % methanol). The products were separated by preparative TLC using Benzene + 1.4 % Methanol as the eluant. The product of Rf 0.4 (30 mg) on crystallisation furnished colourless crystals of tetrahydrophysanol A, m.p. 129°(25 mg). $\nu_{max.}^{(KBr)}$, 3401, 2907, 1710, 1459, 1337, 1313, 1276, 1256, 1199, 1182, 1139, 1116, 1071, 1037, 991, 971, 874, 714 cm^{-1}, PMR : ppm. 0.75, 0.90 (3H each s, 2 x quaternary C$\underline{H}_3$), 3.9 (1H, dm, J = 10 Hz, -C$\underline{H}$-OH), 4.6 (1H, m, -C$\underline{H}$-O.CO-$C_6\underline{H}_5$), 7.5-7.8, 8.1-8.35 (3H and 2H respectively, $C_6\underline{H}_5$). Found : C, 73.49; H, 10.01, $C_{36}H_{54}O_4$ requires : C, 78.54; H, 9.81 per cent. M^+550.

The product of Rf 0.12 (250 mg) crystallised out as fine needles of dihydrophysanol A from chloroform-methanol, m.p. 232-33°, (220 mg) $[\alpha]_D$ = 68° (C 1 %, chloroform) $\lambda_{max.}^{EtOH}$ 235 nm, $\nu_{max}^{(KBr)}$, 3480, 3035, 3010, 2985, 2860,

1720, 1680, 1630, 1604, 1580, 1460, 1380, 1332, 1318, 1276, 1225, 1211, 1132, 1122, 1112, 1076, 1032, 1009, 979, 935, 872, 843, 829, 813, 714 cm^{-1}; PMR ; ppm. 0.62, 0.93 (3H each s, 2 x quaternary $C\underline{H}_3$); 3.63 (1H, dm, J = 10 Hz, $-\overset{|}{C}\underline{H}-OH$); 4.78 (1H, m, $-\overset{|}{C}\underline{H}-O-COC_6H_5$), 5.8 (1H, q, J = 1.5 Hz, $-\overset{|}{C}=C\underline{H}-CO-$); 7.65, 7.81 (3H, m and 2H, dd, J = 7 and 2 Hz respectively, $-C_6\underline{H}_5$). Found : C, 78.67; H, 9.47. $C_{36}H_{52}O_4$, requires : C, 79.83; H, 9.43 per cent, M^+543.

The hydrogenation of physanol A in ethylacetate in presence of platinum catalyst at atmospheric pressure for 6 hours, after a usual work up furnished only dihydro-physanol A.

Hydrolysis of physanol A : Physanol A (300 mg) was stirred in 0.2N methanolic potassium hydroxide (25 ml) at room temperature for about 43 hours. The reaction mixture was diluted with small amount of water and then methanol was evaporated under reduced pressure. The aqueous concentrate was extracted with ether. The ethereal layer was washed thrice with dilute hydrochloric acid solution, sodium hydrogen carbonate solution and water in a sequence, dried and evaporated to yield a neutral product. The aqueous alkaline phase was acidified with dilute HCl and extracted with ether. The organic phase was washed with water, dried

and evaporated to dryness. The acidic residue was crystallised from water furnishing white crystalline compound, m.p. 121-22° which was identified as benzoic acid by comparison of its m.p, IR and TLC with those of the authentic sample.

The neutral product obtained above showed two spots on TLC, Rf. 0.24 and 0.36 (Benzene + 8 % methanol). These products were designated as debenzoylphysanol A and A_1 in order of increasing Rf values. The neutral mixture (250 mg) was chromatographed over neutral alumina (18 g), thirty fractions of 10 ml each were collected (Table 2).

TABLE 2 : Chromatography of the neutral mixture.

Fraction Number	Eluant	Weight (g)	TLC
1- 6	Benzene : Chloroform (3:1)	-	-
7-14	Benzene : Chloroform (1:1)	0.155	Debenzoyl-physanol A_1
15-19	Benzene : Chloroform (1:1)	0.045	Debenzoyl-physanol A_1.A
20-30	Benzene : Chloroform (1:3)	0.03	Debenzoyl-physanol A

The fractions 7-14 (Table 2) were mixed, concentrated and the residue (155 mg) was crystallised from petrol ether-methanol to give pure debenzoylphysanol A_1, m.p. 210-11°, 120 mg.

The fractions 20-30 (Table 2) on evaporation furnished a residue (30 mg) which on crystallization gave crystalline substance debenzoylphysanol A, m.p. 208-9°, from methanol.

Debenzoylphysanol A : It was obtained as colourless crystalline needles from methanol, m.p. 208-9°..It gave positive Liebermann-Burchard test, $\lambda_{max.}^{EtOH}$ 245 nm, (ϵ 12, 250), $\nu_{max.}^{KBr}$ 3533, 3250, 2950, 1670, 1633, 1433, 1383, 1300, 1250, 1140, 1050, 997, 980, 890, 870, 770 cm^{-1}. NMR : ppm. 0.63, 0.86 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$); 1.05, 1.06 (2 x 3H, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 3.18 (1H, m, $-C\underline{H}$ OH); 3.85 (1H, dm, J = 10 Hz, $-C\underline{H}OH$); 4.93, 5.03 (1H each, s, $-\overset{|}{C} = C\underline{H}_2$); 5.76 (1H, q, J = 1.5 Hz, $-\overset{|}{C} = C\underline{H}-CO-$). Found : C, 65.59; H, 10.51, $C_{29}H_{46}O_3$ requires : C, 65.61; H, 10.4 per cent, M^+ 442.

Debenzoylphysanol A_1 : It was crystallised from petrol ether/methanol as colourless needles, m.p. 210-11°. It gave positive Liebermann-Burchard test, λ_{max}^{MeOH} 203 nm, (ϵ,8, 142), $\nu_{max.}^{(KBr)}$: 3445, 3250, 2933, 1703, 1638, 1467, 1382, 1310, 1248, 1212, 1187, 1050, 998, 970, 930, 897, 650, 604 cm^{-1},

PMR ; ppm. 0.64, 0.90 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$); 1.05, 1.1 (2 x 3H, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 3.0 (2H narrow m, $-\overset{|}{C} = C-C\underline{H}_2-CO-$); 3.20 (1H, m, $-C\underline{H}OH$); 3.86 (1H, dm, J = 10 Hz, $-C\underline{H}OH$); 4.9, 5.0 (1H each s, $>C = C\underline{H}_2$) Found ; C, 65.58; H, 10.49, $C_{29}H_{46}O_3$ requires ; C, 65.61; H, 10.4 per cent, M^+442.

Oxidation of debenzoylphysanol A_1 ; Debenzoylphysanol A_1 (30 mg) was dissolved in acetone and 3 N chromic acid (Jones' reagent) was added dropwise until an orange colour persisted. After allowing to stand for 10 minutes at room temperature, acetone was removed under reduced pressure and the residue was diluted with water and extracted with ether. The ether solution was washed thoroughly with dilute solution of sodium hydrogen carbonate and water, dried and evaporated. The residue crystallised from methanol as white crystalline needles, m.p. 191°, $\lambda_{max.}^{EtOH}$ 217 nm, $\nu_{max.}^{(KBr)}$; 2950, 2806, 1711, 1633, 1456, 1383, 1360, 1325, 1308, 1243, 1210, 1053, 1010, 973, 892 cm^{-1}; PMR ; ppm. 0.87, 1.12 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$); 3.03 (2H, m, $-\overset{|}{C} = C-C\underline{H}_2-CO-$); 3.23 (2H, s, $-\overset{|}{C}O-C\underline{H}_2-$); 4.88, 5.05 (1H each s, $-\overset{|}{C} = C\underline{H}_2$). Found ; C, 66.19; H, 9.41; $C_{29}H_{42}O_3$ requires ; C, 66.21; H, 9.36 per cent, M^+439.

Sodiumborohydride reduction of physanol A ; To a solution of physanol A (100 mg) in dioxane (15 ml) was added sodiumborohydride (100 mg) and the reaction mixture was

stirred at room temperature for 5 hours. After partial removal of the solvent by distillation under reduced pressure, the mixture was poured over crushed ice, acidified with dilute sulphuric acid and extracted with benzene. The benzene extract was thoroughly washed with water till neutral to litmus paper, dried and evaporated to dryness. The residue was purified by chromatography over silica gel. The benzene + 0.2 % methanol eluate, on crystallisation from acetone, yielded fine colourless needles, m.p. 221-22°. $\nu_{max}^{(KBr)}$, 3460, 2950, 1721, 1637, 1451, 1381, 1250, 1211, 1151, 1094, 1063, 1043, 1027, 985, 957, 935, 918, 891, 874, 859, 843, 815, 793, 714 cm^{-1}; PMR : ppm. 0.65, 1.13 (3H each, s, 2 x quaternary $C\underline{H}_3$); 2.31 (1H, dm, J = 10 Hz, $-C\underline{H}OH$); 4.26 (1H, t, J = 5 Hz, $-C\underline{H}OH$); 4.76 (1H, m, $-C\underline{H}OH$); 4.38, 5.0 (1H each, s, $-\overset{|}{C} = C\underline{H}_2$); 5.56 (1H, d, J = 5 Hz, $-\overset{|}{C} = C\underline{H}-$); 7.61, 8.18 (3H, m and 2H, dd, J = 7 and 2 Hz respectively, $C_6\underline{H}_5$); Found : C, 78.79; H, 9.51, $C_{36}H_{52}O_4$ requires : C, 78.83; H, 9.48 per cent, M^+548.

Attempted Wolff-Kishner Reduction (Barton's modification) of 11-Ketophysanol A : A mixture of diethylene glycol (5 ml redistilled), complete anhydrous hydrazine (2 ml) and sodium (80 mg) was heated at 130° for 30 minutes. The sodium (80 mg) was heated at 180° for 30 minutes. The solution was then cooled, 11-Ketophysanol A (200 mg) added

quickly and the solution refluxed overnight. The temperature was then raised to 210° by distilling some of the hydrazine and the solution refluxed at this temperature for 24 hours. The reaction mixture was diluted with water and extracted with chloroform. The chloroform layer was washed thoroughly with water, dried over sodium sulphate and freed of solvent. A complicated mixture was thus obtained (TLC) from which no product could be isolated.

Attempted Wolff-Kishner Reduction (Nagata's modification) of 11-Ketophysanol A : A mixture of 11-ketophysanol A (250 mg), hydrazine (93 %; 6 ml), hydrazine dihydrochloride (1 g) and triethylene glycol (10 ml) was heated at 130° for 7 hours. After addition of potassium hydroxide (1.1 g), the temperature was slowly raised and the mixture was heated at 220° for 3 hours. The reaction mixture was then worked up as usual. A complicated mixture of products obtained.

Catalytic hydrogenation of physanol A in presence of $HClO_4$: Physanol A (100 mg) was dissolved in glacial acetic acid (80 ml) containing 0.012 % of $HClO_4$ and hydrogenated in the presence of platinum catalyst at the atmospheric pressure. The reaction mixture was filtered and freed of the acetic acid at 50° under reduced pressure. The residue was diluted with water and extracted with ether. The ethereal layer was washed with aqueous $NaHCO_3$ solution

and then with water, dried and evaporated. The residue was found to be a mixture of dihydrophysanol A and a number of other unidentified products on TLC.

Attempted preparation of a thioketal of O-acetylphysanol A :

(a) A mixture of O-acetylphysanol A (200 mg), ethanedithiol (0.2 ml) and BF_3 etherate (0.15 ml) was stirred for two hours at room temperature. The reaction mixture was taken up in ether (20 ml) and washed with water and sodium bicarbonate solution. Excess ethanedithiol was removed by addition of a saturated ethereal solution of $HgCl_2$. The precipitate formed was filtered and the filtrate was washed with water, concentrated KI solution and saturated NaCl solution in a sequence, dried and evaporated. The resulting product was an inseparable mixture of a large number of products.

(b) To a solution of O-acetylphysanol A (100 mg) in dry benzene (50 ml) was added ethanedithiol (0.2 ml) and p-toluenesulphonic acid (10 mg) and the reaction mixture was refluxed on oil bath for 9 hours with azeotropic removal of water. The solvent was then partly removed from the reaction mixture under reduced pressure, followed by addition of water. The benzene layer was washed with dilute $NaHCO_3$ solution, followed by water, dried and evaporated. The TLC of the residue showed it to be a mixture consisting mainly of the starting material and a number of other products.

Catalytic hydrogenation of physanol A : Physanol A (150 mg) was dissolved in ethylacetate (20 ml) and hydrogenated in the presence of palladium on carbon (5 %) catalyst under pressure (25-30 lbs) for 4 hours. The reaction mixture after usual work up yielded dihydrophysanol A.

Compound B (Physanol B) : Physanol B crystallised from chloroform-methanol as colourless needles, m.p. 232-33° $[\alpha]_D$ = 68° (C, 1 %, chloroform), Rf 0.35 (Benzene + 2 % methanol). It gave a positive Liebermann-Burchard test; λ_{max}^{EtOH} 235 nm; $\nu_{max.}^{(KBr)}$: 3480, 3035, 3010, 2985, 2360, 1720, 1680, 1630, 1604, 1580, 1460, 1380, 1276, 1112, 1076, 1032, 1010, 970, 935, 872, 714 cm^{-1}; PMR : ppm. 0.62, 0.91 (3H each, s, 2 x quaternary $C\underline{H}_3$); 1.07, 1.08 (3H each, d, J = 7 Hz, 2 x secondary $C\underline{H}_3$); 3.65 (1H, dm, J = 10 Hz, $-C\underline{H}-OH$); 4.78 (1H, broad m, $-C\underline{H}-OCO-$); 5.81 (1H, q, J = 1.5 Hz $-C=C\underline{H}-CO-$); 7.53, 8.1 (3H, m, and 2H, dd, J = 7 & 2 Hz respectively, $C_6\underline{H}_5$). Found : C, 78.77; H, 9.47; $C_{36}H_{52}O_4$ requires : C, 78.83; H, 9.49, per cent; M^+ 540, 426 (M-122), 285 (M-122-141), 243 (285-42).

Mono-O-acetylphysanol B : Physanol B (90 mg) was dissolved in dry pyridine (0.5 ml) and acetic anhydride (0.5 ml) and allowed to stand overnight. The reaction mixture was then worked up as usual. The product was purified by chromatography on alumina and crystallised

from chloroform-methanol as colourless needles, m.p. 232°, $\nu_{max.}^{(KBr)}$; 2967, 2924, 1730, 1724, 1699, 1647, 1621, 1604, 1462, 1397, 1340, 1280, 1252, 1211, 1192, 1151, 1119, 1082, 1035, 981, 875, 830, 817, 717, 692 cm^{-1} PMR ; ppm. 0.62, 0.93 (2 x 3H, s, 2 x quaternary $C\underline{H}_3$); 2.05 (3H, s, $-OCOC\underline{H}_3$); 4.76 (1H, broad m, $-C\underline{H}-OCOC_6H_5$); 5.1 (1H, dm, J = 10 Hz, $-C\underline{H}-OAc$); 5.78 (1H, q, J = 1.5 Hz, $-C = C\underline{H}-CO-$); 8.15, 7.58 (2 t, dd, J = 7 and 2 Hz; 3H, m, respectively, $C_6\underline{H}_5$); Found : C, 77.19; H, 9.22; $C_{33}H_{54}O_5$ requires : C, 77.28; H, 9.15 per cent, M⁺590.

Mono-O-benzoylphysanol B : Physanol B (60 mg) was treated with benzoylchloride (0.5 ml) in dry pyridine (0.5 ml) and the reaction mixture was allowed to stand overnight. It was worked up in usual manner and purified by chromatography on alumina. The product was crystallised from chloroform-methanol as colourless needles, m.p. 230-31°, $\nu_{max.}^{(KBr)}$; 2967, 2882, 1727, 1699, 1634, 1642, 1621, 1604, 1453, 1397, 1326, 1280, 1229, 1192, 1119, 1080, 1035, 981, 875, 716, 692 cm^{-1}, PMR ; ppm. 0.63, 0.93 (2 x 3H each, s, 2 x quaternary $C\underline{H}_3$); 4.78 (1H, m, $-C\underline{H}-OCOC_6H_5$); 5.78 (1H, q, J = 1.5 Hz, $-C = C\underline{H}-CO-$); 5.35 (1H, dm, J = 10 Hz, $-C\underline{H}-O-CO-C_6H_5$); 7.51-7.8, 8.05-8.35 (6H and 4H respectively; 2 x $C_6\underline{H}_5$). Found : C, 79.06; H, 8.84; $C_{43}H_{56}O_5$ requires : C, 79.14; H, 8.58 per cent, M⁺652.

Compound C (β-sitosterol) : It was crystallised from chloroform-methanol as colourless needles, m.p. 136-37°, Rf 0.12 (Benzene + 2 % methanol), $[\alpha]_D$ = -30° (chloroform) with acetic anhydride-sulphuric acid it gave the colour sequence, purple ⟶blue⟶ green.

Acetylation : Compound C (50 mg) on reaction with acetic anhydride and dry pyridine gave an acetate which was crystallised from alcohol, m.p. 124°.

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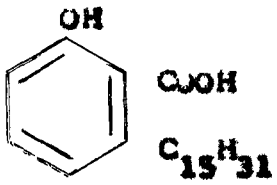
DISCUSSION OF CHAPTER II

(Chemical Investigation of *Anacardium gigantenum* Hancock ex Engl.)

The plants belonging to the family Anacardiaceae, like Semecarpus anacardium Linn., Anacardium occidentale Rhus vernicefera, Rhus succedanea.etc. are widely distributed in the eastern countries viz. China, Japan, Burma, Indo-China etc. They are resin bearing plants and the resins have been used for the manufacture of lacquers and varnishes. In India there are a few of these plants like Anacardium occidentale L. and Semecarpus anacardium Linn. The resin known as cashew nut (A. occidentale L.) shell oil is exported, but no figures are available about the plantation and production of Semecarpus anacardium Linn. However, the resin from S. anacardium Linn. is also being used in industry.

The resinous pericarp juice of the cashew nut (Anacardium occidentale L.) was investigated in 1847 by Staedeler[1] who reported the isolation of a non-acidic, vesicant principle Cardol and anacardic acid, which was later purified by Ruhemann and Skinner[2]. Smit[3] studied the constitution of anacardic acid and later on Pillay[4] proposed the structure of tetrahydroanacardic acid as o-pentadecyl salicylic acid which was supported through synthetic studies of Gokhale, Patel and Shah[5] as well as

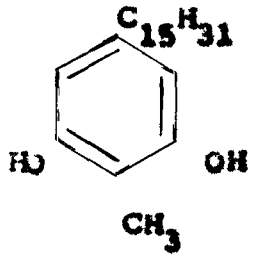
Baker and Hack[6]. Recently, Tyman et al.[7] have given a new synthesis for tetrahydroanacardic acid and finally confirmed its structure (I) as 6-pentadecylsalicylic acid.



(I)

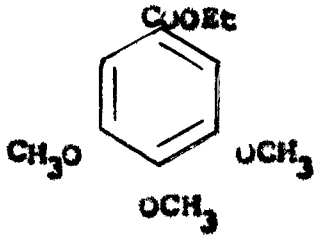
Baker and Hack[6] also studied the vesicant principle, cardol and considered it to be allied to bhilawanol and urushiol.

Later Siddiqui et al.[8] made a systematic study of Anacardium occidentale L. and further isolated three compounds namely, Kajin, $C_{19}H_{20}O_9 \cdot 1\frac{1}{2}H_2O$, m.p. 174°, gallic acid, m.p. 241°, and Kajidin, which was proved to be ellagic acid[9]. Recently, Tyman[10] isolated a new phenol (II) from the cashew nut shell liquid and identified it as a C-methylated product of cardol.



(II)

Seri[11] isolated β-sitosterol from the bark of A. occidentale L. and Subramanian[12] isolated ethyl 3,4,5-trimethoxy-benzoate (III) from the gum of A. occidentale L.

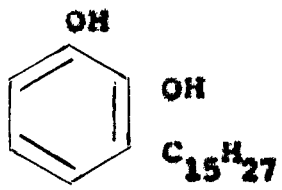


(III)

From the flowers of A. occidentale L. ethylgallate, quercetin, hyperoside and M-digallic acid have been isolated by Subramanian et al.[13], who also found β-sitosterol, ethylgallate, hyperoside and methylgallate in the tender leaves[14] and β-sitosterol, (-)-epicatechin and (+)-catechin in the testa of the commercial variety of cashew nuts available in Kerala.

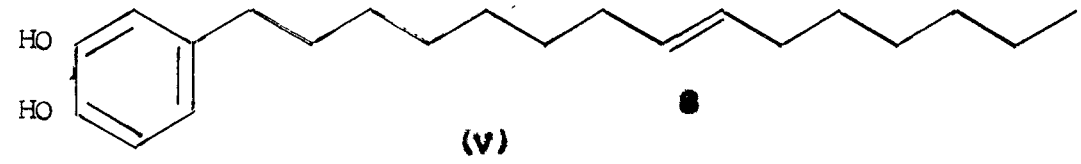
The well known marking nut, Semecarpus anacardium, Linn., (N.O. Anacardiaceae; Hindi : Bhilawan) has found use in the Indian system of medicine in the treatment of rheumatism[15], epilepsy and nervous debility and externally for leprosy, eczema and other skin diseases. Naidu[16] reported the isolation of a fixed oil, catechol, anacardol and anacardic acid from the kernels of Semecarpus anacardium

Linn. Pillay and Siddiqui[17] isolated an unidentified monophenol called semo-carpol and a catechol derivative named bhilawanol, the constitution of which was established (IV) through the oxidation of its tetrahydro derivative to palmitic acid and to catechol through thermal degradation.



(IV)

The $C_{15}H_{27}$ chain was said to be long unsaturated side chain with two double bonds. Rao and Row[18], however, gave the structure of bhilawanol as (V) through the oxidation of methylated bhilawanol to n-heptanic acid and an aromatic acid proved to be 8-(3'4-dimethoxy-phenol)-octanoic acid by a study of the NMR and Mass spectra of its methyl ester (VI)



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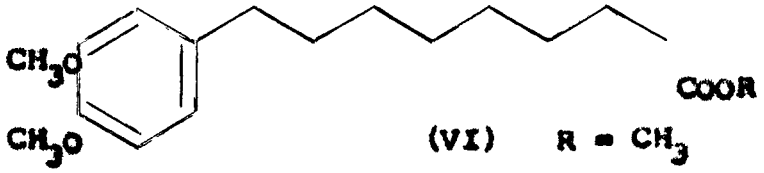
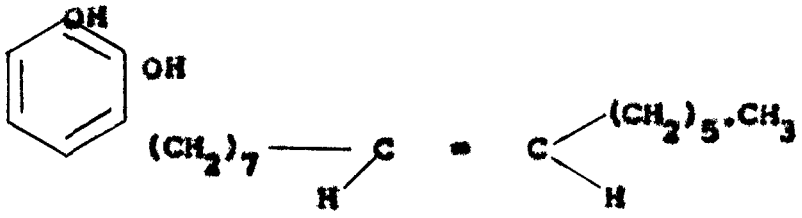


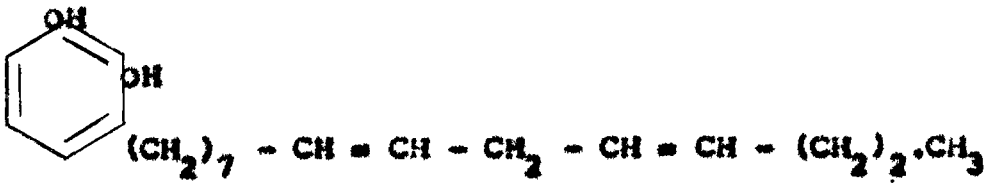
(VI) R = CH_3

In more recent years, Govindachari et al.[19] have shown that bhilawanol is a mixture of two phenolic compounds consisting mainly of 1,2-dihydroxy-3-(pentadecyl-8') benzene (VII),



(VII)

and 1,2-dihydroxy-3-(pentadecyldienyl-8',11')-benzene (VIII).



(VIII)

Khare et al.[20] have also reported the isolation of anacardic acid from Semecarpus anacardium Linn. and mentioned its sodium salt to be a potent anthelmintic agent, its activity being higher than that of piperazine at the same concentration.

In view of the interest shown in laccol type of compounds mentioned above, the author carried out chemical investigations on the fruits of Anacardium gigantum

Hancockex Engl. (N.O. Anacardiaceae) growing in South America and the results are described below.

Isolation of Anagigantic acid

The pericarp of the mature nuts of Anacardium giganteum Hancock ex Engl. were extracted with n-hexane. Concentration of the extracts under reduced pressure yielded a viscous oily mass which gave a heavy deposit of a crystalline mass on cooling. The crystallizate was fractionated by making use of water miscible and water-immiscible solvents without the use of alkalies or acids, when finally a colourless compound was obtained which crystallised from n-hexane in the form of stars and aggregates of silky needles, m.p. 81°, $C_{13}H_{28}O_3$, λ max. 220, 246, 312 nm.

The compound, provisionally designated as anagigantic acid, gave a violet blue colouration with ferric chloride indicating the presence of a phenolic group. It was soluble in the usual organic solvents and was also soluble in dilute sodium bicarbonate solution, indicating the presence of a carboxylic group. The IR spectrum of anagigantic acid manifested the presence of an aromatic nucleus (3040, 1535, 1484, 709 cm^{-1}), CH_3-CH_2-(2950, 2865, 1458, 1391, 732 cm^{-1}), Ar-COOH (3300, 2330, 1681 cm^{-1}).

The PMR spectrum of anagigantic acid exhibited signals for a terminal $-CH_2-CH_3$ group (3H, t, J = 7 Hz at 0.88 ppm), $-(CH_2)_9-$ (13H, s, with a broad base at 1.23 ppm), $-CH_2-CH_2-Ar$ (2H, t, J = 7 Hz at 3.011 ppm), three adjacent aromatic protons (1H, dd, J = 7 and 1.5 Hz at 6.93 ppm and 1H, t, J = 7 Hz at 7.43 ppm), hydrogen bonded hydroxyl protons (2H, broad s, at 11.01 ppm).

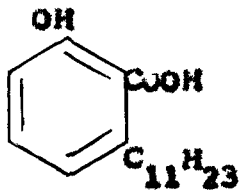
Methylation of the compound with diazomethane yielded a colourless viscous liquid, $C_{19}H_{30}O_3$, insoluble in bicarbonates, apparently due to the methylation of the carboxylic group in the process.

Methylanagigantate obtained above, however, gave a violet colouration with ferric chloride, indicating the presence of a free phenolic group and it was therefore, further methylated with dimethyl sulphate and 15 per cent aqueous potassium hydroxide. The precipitated oily mass was worked up in the usual manner when a colourless viscous oil, $C_{20}H_{30}O_3$, was obtained in an almost quantitative yield which gave no colouration with ferric chloride and was found to be insoluble in dilute alkalies. Its IR spectrum showed the presence of an aromatic ester grouping (1743, 1276 cm^{-1}), aromatic nucleus (3030, 1604 cm^{-1}), CH_3-CH_2-(2865, 2933, 1479, 1199 cm^{-1}) but no absorption band for any hydroxyl grouping.

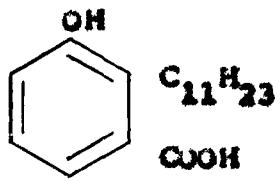
From the above observations it was obvious that anagigantic acid was an aromatic carboxylic acid having a long residual $C_{11}H_{23}$- side chain and a phenolic group. The non-reducibility of anagigantic acid with hydrogen in presence of platinum or palladium catalyst also supported the view that the aliphatic side chain was saturated. In order to further establish the nature of $C_{11}H_{23}$-side chain, attempts were made to bring about an oxidative cleavage under various conditions. Initial attempts to oxidise anagigantic acid with alkaline potassium permanganate in aqueous medium, alkaline potassium permanganate in acetone, concentrated nitric acid did not yield any crystalline product. Finally, its oxidation with potassium permanganate was possible in aqueous pyridine which resulted in the isolation of lauric acid, m.p. 44°. The identity of the product was established through its analytical data, a comparison of its parallel and mixed melting point and superimposable IR spectra with an authentic sample. The formation of this acid must have been due to the splitting of the aromatic nucleus and thus the simplified formula for anagigantic acid could be written as $(C_6H_3).(OH).(COOH).(C_{11}H_{23})$.

In order to fix the relative positions of the hydroxyl, carboxyl and the $C_{11}H_{23}$-side chain with respect to each other in the benzene nucleus, the methyl ether of

methylanagigantate was oxidised with potassium permanganate in aqueous pyridine solution yielding an acid which on sublimation in high vacuo gave o-methoxyl phthalic anhydride. Its identity was established by a comparison of parallel and mixed melting points with an authentic sample and superimposable IR spectra. This indicated that the carboxyl and the undecyl chain in anagigantic acid are in adjacent positions to each other. The position of the hydroxyl group remained undecided as o-methoxylphthalic anhydride in the above oxidation could be obtained irrespective of whether the hydroxyl group in anagigantic acid is in ortho position to the carboxyl group or to the undecyl chain as shown in (IX) and (X).



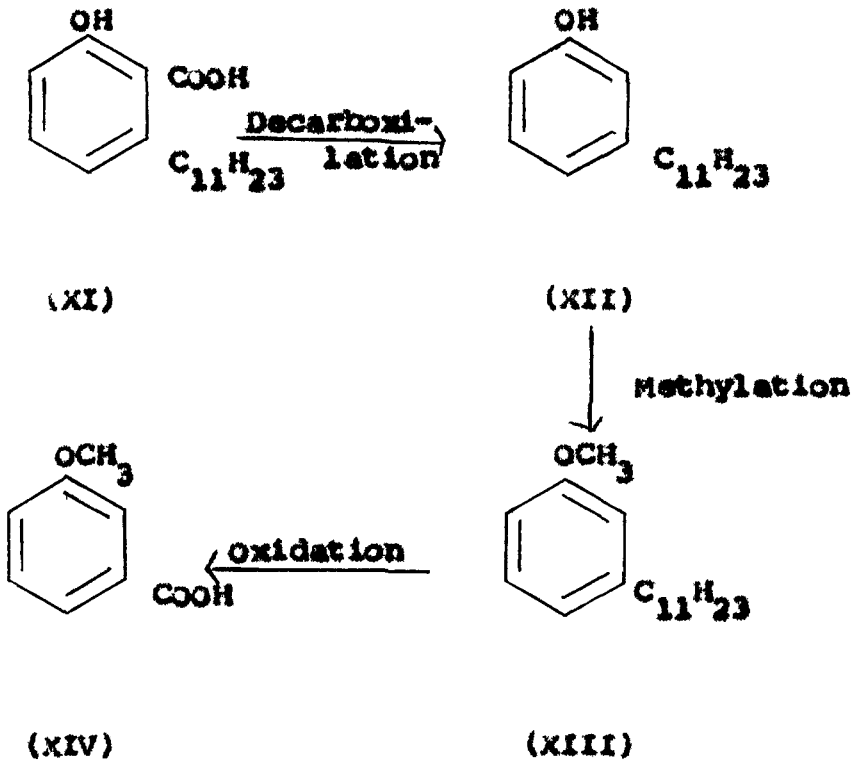
(IX)



(X)

In order to settle this point anagigantic acid was decarboxylated with sodalime to the corresponding phenol, anagigantol (XII), $C_{17}H_{28}O$, which was converted to its methyl ether (XIII), $C_{18}H_{30}O$, by dimethyl sulphate and

aqueous potassium hydroxide solution (15 per cent). The methyl ether on oxidation with aqueous potassium permanganate in pyridine solution yielded m-methoxyl benzoic acid (XIV), the identity of which was established



through its mixed melting point and superimposable IR spectra with an authentic sample. This showed that the undecyl chain and the hydroxyl function in anagigantol are in meta position to each other and therefore the structure of anagigantic acid could thus be written as (XI) as in the above scheme. Recently, Tyman and Durrani[7] have synthesised anagigantic acid and confirmed its structure as (XI).

It is interesting to note in this connection that in the laccol type of compounds isolated so far from the other members of the Anacardiaceae family, a saturated $C_{11}H_{23}$-side chain has not been hitherto met with-a C_{15} or a C_{17} unsaturated chain being of normal occurrence.

EXPERIMENTAL OF CHAPTER II

The pericarp (1 kg) of the mature nuts of Anacardium gigantcum Hancock ex Engl. were repeatedly percolated with petrol ether (60-80°) at the ordinary temperature and the combined extracts were concentrated in vacuo below 50°. The dark brown concentrate was kept in the cold for a few days when a deposit of crystalline material was obtained which after maceration with cold petrol ether was filtered, m.p. 63-74° (37 g).

Anagigantic acid : The crude product, as obtained above was repeatedly crystallised from n-hexane when it finally yielded the pure product in the form of stars and aggregates of silky needles, m.p. 81°. Anagigantic acid gave a violet blue colouration with ferric chloride and was soluble in dilute sodium bicarbonate solution as well as in dilute alkalies. It was soluble in the normal organic solvents. On drying to constant weight in vacuo over P_2O_5 at 50° it gave $\lambda_{max.}^{EtOH}$ 220, 246, 312 nm ; $\nu_{max.}^{(KBr)}$: 3484, 3040, 2950, 2865, 2571, 1681, 1629, 1595, 1494, 1458, 1391, 1314, 1256, 1225, 1178, 1138, 892, 834, 817, 730, 753, 732, 709 cm^{-1}, PMR : ppm. 0.88 (3H, t, J = 7 Hz, terminal $-CH_2-CH_3$),

1.28 (18H, s, with a broad base, $-(CH_2)_9$; 3.011 (2H, t, J = 7 Hz, $-CH_2-\underline{CH}_2-C_6H_5$); 6.8, 6.93 and 7.43 (1H, dd, J = 7 and 1.5 Hz, 1H, dd, J = 7 and 1.5 Hz and 1H, t, J = 7 Hz respectively, three adjacent aromatic protons); 11.01 (2H, broad s, hydrogen bonded hydroxyl protons). Found : C, 73.9; H, 9.9; eq. wt. (titration), 297; Ag-salt, 293; active-H, 0.66; O CH_3 (Zeissel), nil; M.Wt (Rast), 292; $C_{19}H_{28}O_3$ requires : C, 73.9; H, 9.6; M.wt, 292; active-H (for two), 0.66 per cent.

Methylation of anagigantic acid : Anagigantic acid (1.5 g) dissolved in 100 ml of ether was treated with an excess of ethereal diazomethane so that a purmanent yellow colour was obtained. There was vigorous evolution of nitrogen and the solution was kept overnight and again treated with a few ml of diazomethane to a permanent yellow colour and kept overnight. Finally, the solvent was distilled off on the water bath when a colourless viscous liquid was obtained which was dried in vacuo at the ordinary temperature to constant weight over phosphorous pentoxide.

Methyl anagigantate was insoluble in sodium bicarbonate solution and gave a violet colouration with ferric chloride. The dried sample on analysis gave C, 74.5; H, 9.91; active-H, 0.32; OCH_3, 10.35; $C_{19}H_{30}O_3$ requires : C, 74.5; H, 9.8; active H(for one), 0.33; OCH_3(for one), 9.6 per cent.

Methylation of methyl anagigantate : Methyl anagigantate (1 g) was dissolved in 100 ml of 15 per cent potassium hydroxide solution and dimethyl sulphate (25 ml) was added dropwise with continuous stirring. After the complete addition of dimethyl sulphate, the reaction mixture was made alkaline with the addition of a further quantity of alkaline solution (15 per cent), warmed on the water bath and extracted with ether. The ethereal solution was washed with water, dried over sodium sulphate and freed of the solvent. The residual colourless viscous liquid was obtained in an almost quantitative yield, gave no colouration with ferric chloride and was found to be insoluble in dilute alkalies. The sample dried in vacuo over P_2O_5 gave $\nu_{max.}^{(neat)}$: 3030, 2933, 2865, 1743, 1604, 1479, 1276, 1199, 1112, 1080, 970, 884, 812 cm^{-1}, Found : C, 75.0; H, 10.1; active-H, nil; OCH_3, 17.3; $C_{20}H_{32}O_3$ requires : C, 75.0; H, 10.0; OCH_3 (for two), 19.3 per cent.

Oxidation of anagigantic acid : Anagigantic acid (2 g) was dissolved in 30 ml of pyridine and the solution was diluted with 10 ml of water. The solution was heated on the water bath with slow addition throughout of small quantities of well powdered potassium permanganate (10 g) under vigorous stirring. Initially, the decolourisation was quick but after the complete addition of potassium permanganate there was a

slight permanganate colour and the reaction mixture was further heated for 1 hour. It was cooled and filtered - the residual manganese dioxide precipitate was repeatedly washed initially with acetone and then with boiling water. The solvents were distilled off under reduced pressure and the aqueous residue was cooled and acidified, when 600 mg of an acid was obtained which after repeated crystallisation from alcohol finally gave fine needles, m.p. 44°. After drying to constant weight over phosphorous pentoxide in vacuo it gave C, 71.89; H, 12.12; $C_{12}H_{24}O$ requires : C, 72.00; H, 12.00 per cent.

The identity of the acid with lauric acid was established through its analytical data, a comparison of its parallel and mixed melting points and superimposable IR spectra with an authentic sample.

Oxidation of the methylether of methyl anagigantate : The methyl ether of methyl anagigantate (1.5 g) was dissolved in 20 ml of pyridine and diluted with 10 ml of water. Powdered potassium permanganate (6 g) was added portion wise with continuous stirring and heating of the reaction mixture on the water bath. The oxidation was complete after 2½ hours. Sulphurdioxide was passed through the reaction mixture and the acidic solution was extracted with ether and the ethereal solution was washed with water,

dried over anhydrous sodium sulphate and freed of the solvent. The residue on sublimation in vacuo yielded colourless crystalline prisms, m.p. 160° (o-methoxy phthalic anhydride). Found : C, 60.87; H, 3.83; $C_9H_6O_4$ requires : C, 60.7; H, 3.4 per cent. The identity of the crystalline product was established by a comparison of parallel and mixed melting points with an authentic sample and superimposable IR spectra.

Decarboxylation of anagigantic acid : Anagigantic acid (2.8 g) was heated with sodalime under reduced pressure on the oil bath at 175° and finally under high vacuo when it gave a colourless liquid, anagigantol, which gave a blue colouration with ferric-chloride. Found : C, 82.15; H, 11.53; $C_{17}H_{28}O$ requires : C, 82.2; H, 11.3 per cent.

Methylation of anagigantol : Anagigantol (1.8 g) was methylated with dimethyl sulphate and aqueous potassium hydroxide (15 per cent) solution, as in the case of methylation of anagigantic acid. The methyl ether of anagigantol did not give any colour with ferric chloride. The sample dried over phosphorous pentoxide under vacuo gave C, 81.81; H, 11.72; $C_{18}H_{30}O$ requires : C, 82.44; H, 11.44 per cent.

Oxidation of methylether of anagigantol : The methylether of anagigantol (1 g) was dissolved in pyridine (20 ml) and 10 ml of water. The solution was heated on the water bath and treated portion wise with powdered potassium permanganate (5 g) under continuous stirring. The oxidation was complete after 3 hours. The reaction mixture was worked up in the manner as described in the case of oxidation of methylether of methyl anagigantate. A colourless crystalline substance was obtained in the form of fine needles after crystallisation from water, m.p. 110° (m-methoxylbenzoic acid). The sample dried under vacuo over phosphorous pentoxide gave C, 63.66; H, 5.82; $C_8H_8O_3$ requires : C, 63.15; H, 5.26 per cent.

The identity of the product was established through its mixed melting point and superimposable IR spectra with an authentic sample.

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CONCLUSIONS

I. Theoretical

The "Theoretical" presents the highlights of the recent advances in the analytical techniques applied to the isolation and structure elucidation of the steroids. It also includes the list of stigmastane type of compounds isolated and characterised during the period 1958 to 1971, which have been classified and placed in six tables along with the plants of their origin.

II. Chemical Investigation of *Physalis franchetii* Mast.

The hexane extract of powdered seeds of the plant, on chromatography yielded β-sitiosterol and two unknown sterols designated as physanol A and physanol B. The structure of physanol A and physanol B have been proposed on the basis of physicochemical data and degradative studies.

III. Chemical Investigation of *Anacardium giganteum* Hancock ex Engl.

The hexane extract of the pericarp of the mature nuts yielded a new crystalline phenolic acid, designated as anagigantic acid. The structure of anagigantic acid has been elucidated by the spectroscopic and degradative studies.

LIST OF PUBLICATIONS

1. Chemical Investigation of *Anacardium giganteum*.
   Narendra Kumar Sharma and V.N. Sharma, Indian J. Chem., 4, 99 (1966).

2. Structure of Anagigantic Acid Isolated from *Anacardium gigantenum* Hancock ex Engl., N.K. Sharma and V.N. Sharma, Indian J. Chem., 4, 504 (1966).

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